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PESTICIDE PUBLICATION 613

Environmental Protection Agency
Chamblee, Georgia 30341

20. DETERMINATION OF TRACE QUANTITIES OF ANTICHOLINESTERASE PESTICIDES IN AIR AND WATER

M. Cranmer and A. Peoples. Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida

There is currently a great emphasis on establishing the quality of our environment. Two compartments of the environment which became contaminated with a seemingly endless number of potentially toxic agents are air and water. Since the need for air and water is for the most part excluded from the realm of freedom of personal selection and since any contamination is widely dispersed, it is imperative that appropriate standards, enforced, insure quality.

Organophosphate and carbamate insecticides are two classes of toxic chemicals which find their way into both air and water. Since these compounds are extremely toxic even in low concentrations and since absorption from air and water is efficient, even trace amounts may be of biological significance. There is currently no single multi-residue method which is applicable to all organophosphate or carbamate insecticides, and many of the available procedures lack specificity.[1-3] Several extremely sensitive methods for certain organophosphate insecticides utilize gas liquid chromatography and selective detectors. The most sensitive requires 10^{-10}g of parent compound for accurate determination.[4-6]

If extraction and partition, selective detection or derivative formation are considered as part of the procedure, a multi-residue method cannot be based on a common denominator, for both the organophosphates and carbamates structural types represented are too diverse. An obvious common denominator

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is the ability to inhibit cholinesterase. Quality standards usually are set to limit toxicity--and the toxicity of various anticholinesterase insecticides differs markedly. It is reasonable that screening of environmental samples would be accomplished most expeditiously by indexing the total anticholinesterase activity of a sample, rather than by determining the absolute amounts of the several components present, since it is the anticholinesterase potential of these compounds that determines their toxicity. Samples with unacceptable levels of anticholinesterase activity would then be subjected to additional, more specific, analytical manipulation.

The following method is a modification of a sensitive procedure for the gas chromatographic determination of acetylcholinesterase activity in µl volumes of blood.[7] It is applicable to the screening of large numbers of samples with the confidence that if anticholinesterase components are present in toxicologically significant form, they will be detected, and their collective anticholinesterase activity determined.

METHODS

Apparatus

A MicroTek MT-220 gas chromatograph equipped with a single hydrogen flame ionization detector and a Westronics dual pen recorder were used in this study.

The incubation of the reaction was carried out with a Thermolyne Dri-Bath permanently set at 37°. Components of the assay were added with Oxford pipettors, the reaction was carried out in 5-ml glass-stoppered test tubes and a clinical centrifuge was used to separate the CS_2 and the aqueous layers in the final reaction mixtures.

Reagents

Reagents were obtained from the following sources: 3,3-dimethylbutyl acetate (DMBA) and 3,3-dimethylbutanol (DMB), K & K Laboratories; true cholinesterase, Type I, Sigma Chemical Works; paraoxon, Farben Fabriken-Bayer-A.G; malaoxon, American Cyanamid; carbaryl, Union Carbide; Tween 20^{R} emulsifier, Biscayne Chemical Laboratories. All other reagents were ACS grade.

Preparation of Reagents

A stock solution of buffer was prepared by dissolving 44.73 g of potassium chloride, 4.12 g of sodium barbital, and 0.55 g of potassium monobasic phosphate in 200 ml of water.[8] The working buffer solution was prepared by adding 20 ml of stock buffer solution to 75 ml of water, adjusting the pH to 8.0 with 0.1 N HCl, and diluting the solution to a final volume of 100 ml with water.

To remove butanol impurities from DMBA, 5 parts of DMBA were mixed with 1 part of acetic anhydride. The mixture was kept at 37°C for 24 hours and then washed once with water to remove the acetic anhydride and impurities. A 0.2 M solution was prepared by diluting the DMBA with working buffer and adding emulsifier to a concentration of 0.2%. The pH was then adjusted to pH 8.0 with 5% sodium hydroxide.

For gas chromatographic standards, DMB solutions containing 50, 100, 150, and 200 ng/µl were prepared in water containing 0.1% emulsifier. Two ml of CS_2 were added to 2 ml of each standard solution, and the mixtures were shaken well and centrifuged. The top layer was discarded and an appropriate volume of the CS_2 layer was injected into the gas chromatograph. Standard curves were prepared by plotting DMB concentration versus peak height or peak area.

Concentrated formic acid (88%) was diluted 1:1 with distilled water.

A stock enzyme solution was prepared by adding 3 ml of working buffer solution to 50 µM units of true cholinesterase. Dilutions of 16.6 µM units/ml(A), 3.3 µM units/ml(B), 0.66 µM units/ml(C) and 0.13 µM units/ml(D) were prepared in working buffer solution.

Three solutions of the inhibitor of choice *i.e.* carbaryl (2.0 µg/µl, 201 ng/µl and 20.1 ng/µl), paraoxon (27.5 pg/µl, 2.75 pg/µl and 0.275 pg/µl) and malaoxon (3.15 ng/µl, 315 pg/µl and 31.5 pg/µl) are prepared in ethylene glycol and served as standards for comparison of unknown samples.

A 0.2% bromine water solution is prepared by diluting 0.2 ml bromine with 100 ml of distilled water.

Preparation of Sample

Samples of anticholinesterase compounds in water or ethylene glycol have been found to be compatible with the enzymatic system. Alcohol,

ether, acetone and other organic solvents are not acceptable, unless compared to standard curves prepared in a similar way, since they alter the activity of the enzyme solutions.

Samples of anticholinesterase compounds which require activation, *e.g.*, parathion to paraoxon, must be treated with one part of 0.2% bromine water solution to 10 parts of sample solution for one hour at 37°C. The bromine water residue does not interfere with the acetylcholinesterase reaction.

Chromatography

Column: a glass U-tube, 1/4 in. x 6 ft., was packed with Johns-Manville Chromosorb 101, mesh size 80-100, and conditioned overnight at 250°C. Instrument conditions: column oven, 215°C; detector, 265°C; inlet, 245°C; 300 ml/min; hydrogen, 20 ml/min; nitrogen. 40 ml/min.

Procedure

Standard Curves: Inhibition curves were prepared by incubating for one hour at 37°C the reaction mixtures given in Step I below:

	Composition of Reaction Mixture			
	A	*B*	*C*	*D*
I Enzyme Solution plus Inhibitor Solution	100 µl of A + 10	200 µl of B + 20	500 µl of C + 50	2,000 µl of D + 200
II Reaction Mixture used for GLC Analysis	10 µl	50 µl	250 µl	1,250 µl

To 1 ml of working buffer in a 5 ml glass-stoppered test tube add the amounts of incubated reaction mixtures designated in II and warm to 37°C. Add 0.2 ml of 0.2 M DMBA and incubate at 37°C for 30 minutes. After 30 min., stop the reaction with 0.1 ml of 44% formic acid and add 2 ml CS_2. Shake the tubes vigorously, centrifuge and remove aqueous layer from top by aspiration. Inject an appropriate volume of CS_2 into the gas chromatograph for DMB

determination. Carry a blank, containing all reagents except enzyme solution, throughout the procedure.

Unknown: In Step I above substitute the unknown material for the Inhibitor Solution and follow the same procedure.

Calculation of Inhibition:

$$\% \text{ inhibition} = \frac{a - b}{a} \times 100$$

a = chart units of DMB in the uninhibited sample.
b = chart units of DMB in the inhibited sample.
Standard curves are prepared by plotting per cent inhibition versus inhibitor concentration on semi-log paper. Per cent inhibition of samples are taken from these standard curves.

Calculation of Quantity:

Quantification is accomplished by comparing the inhibition obtained with an unknown sample to that obtained with known concentrations of a reference inhibitor. Results would then be expressed in moles of paraoxon, carbaryl, malaoxon, or any inhibitor of choice.

The procedure is summarized by the flow diagram in Table 44.

RESULTS AND DISCUSSION

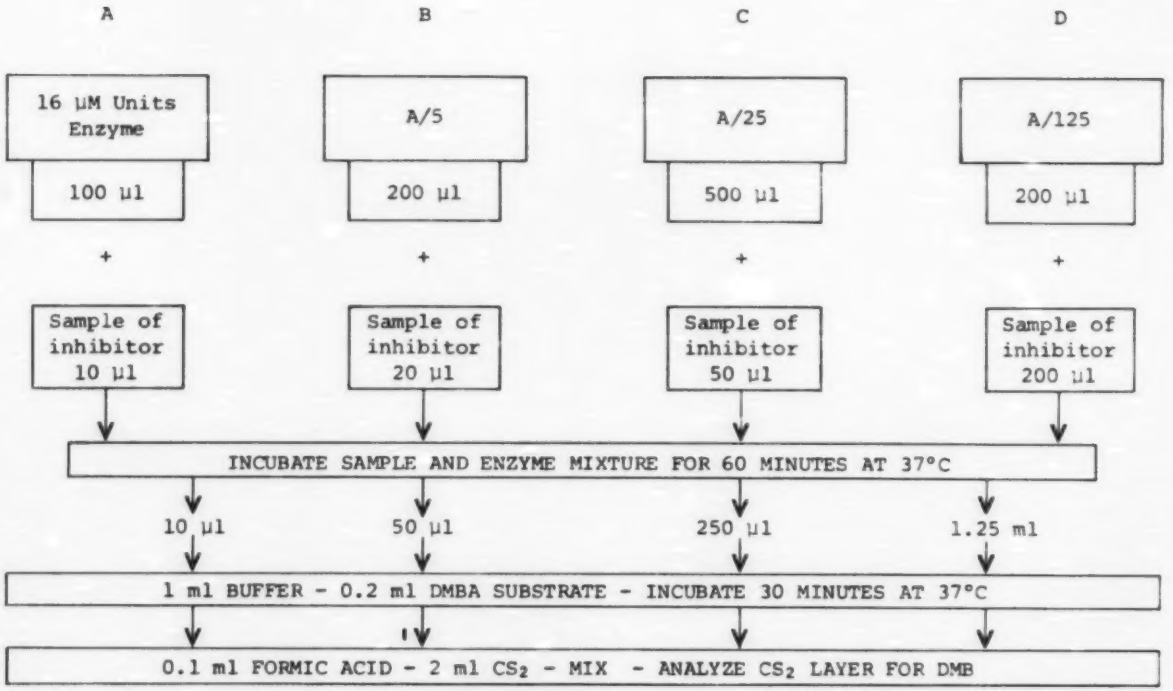
This procedure was based on a GLC method for the determination of the cholinesterase activity of small (µl) amounts of human blood.[7] The selection of 3,3-dimethylbutyl acetate for use as a substrate rather than acetylcholine was determined by several preferred characteristics: (1) decreased non-enzymatic hydrolysis, (2) solubility in a solvent of low hydrogen flame response (CS_2), (3) increased response of 3,3,-dimethyl-1-butanol with respect to choline or acetic acid, (4) satisfactory chromatographic characteristics of both 3,3-dimethylbutyl acetate and 3,3-dimethyl-1-butanol, and (5) similarity to acetylcholine in substrate to enzyme affinity.

Figure 88 illustrates the similarity of the enzyme-substrate fit of 3,3-dimethylbutyl acetate and that of acetylcholine.

Figure 89 illustrates the gas chromatographic characteristics of dimethylbutyl acetate and dimethyl butanol.

Table 44

Procedure Flow Sheet



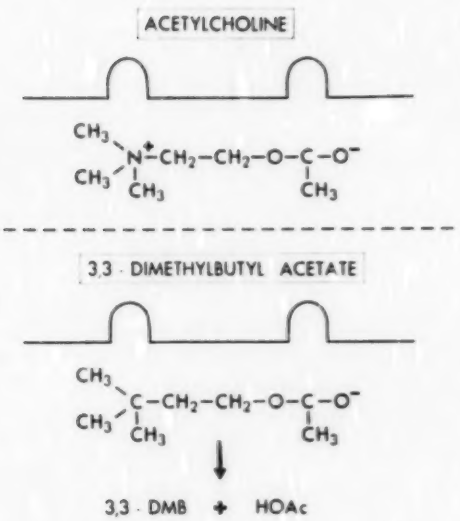


Figure 88. Illustration of similarity of enzyme-substrate fit of 3,3-dimethylbutyl acetate and acetylcholine.

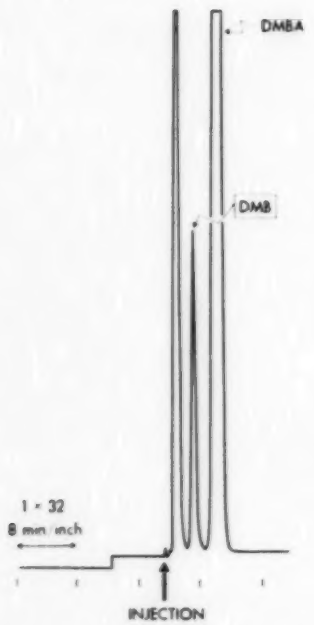


Figure 89. Chromatogram illustrating the resolution of dimethyl butanol (DMB) and dimethylbutyl acetate using a flame ionization detector and Johns-Manville Chromosort 101.

It was anticipated that competitive and non-competitive inhibitors might require different incubation periods with cholinesterase since the mechanism of inhibition is quite different. For example, the phosphorylation of the enzyme by malaoxon proceeds through two steps: (1) intact malaoxon inhibits the enzyme (competitive); (2) malaoxon is hydrolyzed by the enzyme and the enzyme is phosphorylated (slowly reversible) while carbaryl, a carbamate, acts primarily as a competitive inhibitor.

Figure 90 illustrates the inhibition of the four dilutions of enzyme by nano moles of carbaryl per 0.1 ml of each of the enzyme solutions. The

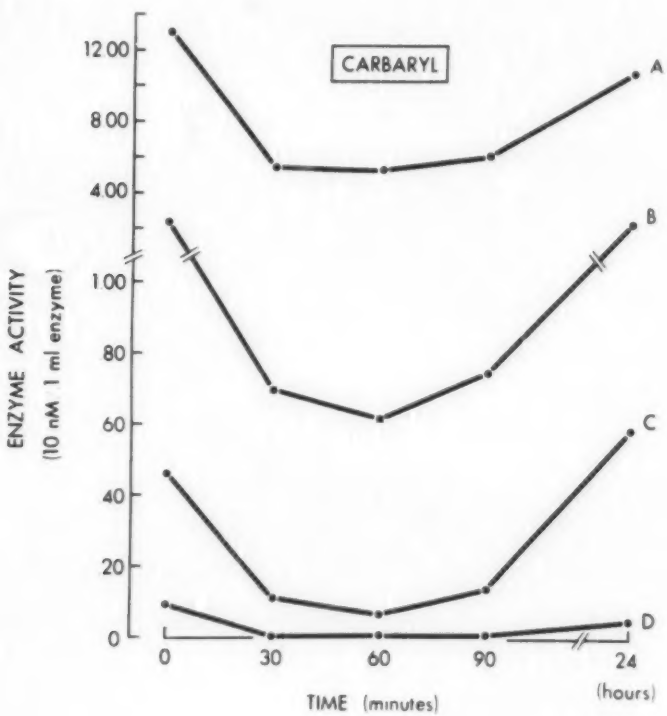


Figure 90. *Inhibition of four activities of true cholinesterase by 10 nmoles of carbaryl/.1 ml enzyme vs time. Enzyme activity: (A) 16.6 µM units/ml, (B) 3.3 µM units/ml, (C) .66 µM units/ml, (D) .13 µM units/ml.*

reduction in activity approaches a maximum in 60 minutes and begins a slow trend toward reactivation after 90 minutes. Malaoxon demonstrated the same trend as illustrated by Figure 91. Similar results

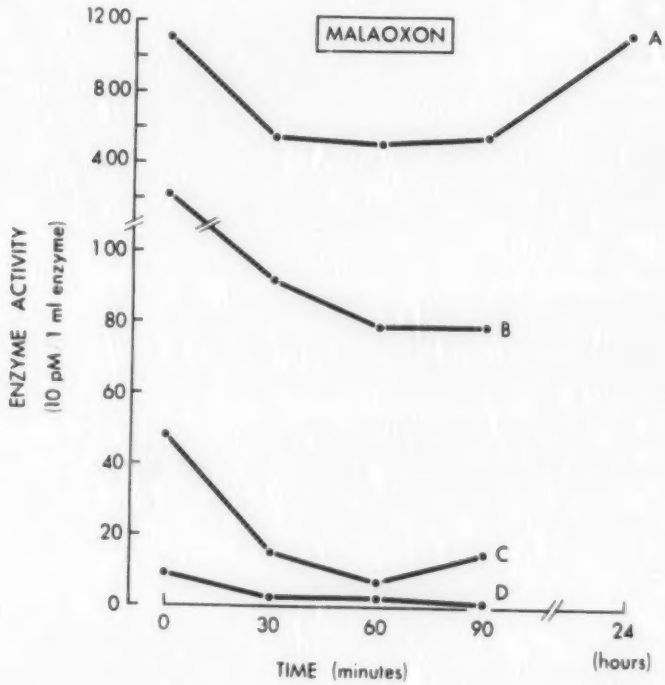


Figure 91. Inhibition of four activities of true cholinesterase by 10 p moles of malaoxon/.1 ml enzyme vs time. Enzyme activity: (A) 16.6 µM units/ml, (B) 3.3 µM units/ml, (C) .66 µM units/ml, (D) .13 µM units/ml.

are obtained for other inhibitors including the very potent inhibitor paraoxon. These observations led to the conclusion that a 60-minute incubation period was sufficient and that there were no problems in analyzing either competitive or non-competitive inhibitors with this assay system.

The conditions of this assay were arranged to screen a wide range of concentrations of cholinesterase inhibitors. Four dilutions differing by

a factor of 5 provide a 125 fold difference in enzyme concentration available for inhibition. By varying the ratios of buffer to enzyme, plus buffer mixture, the dilutions are adjusted to provide an equivalent amount of activity present in each reaction. For example, only 10 µl of the most active enzyme solution (A) is added to 1 ml of buffer while 1.25 ml of the least active (D) solution is used. This compensation results in the same range of reaction product being produced for all initial enzyme concentrations (A, B, C, and D) and greatly simplifies the GLC determinative step. However, since the kinetics of the inhibition of this enzyme depend on the concentration of the enzyme and inhibitor, the different enzyme concentrations do not produce four identical reaction systems. There is, however, a clear relationship between the different enzyme dilutions and the amount of inhibition which results at each inhibitor concentration.

Figure 92 illustrates the inhibition of each of the four enzyme dilutions produced by three paraoxon concentrations differing by a factor of 10.

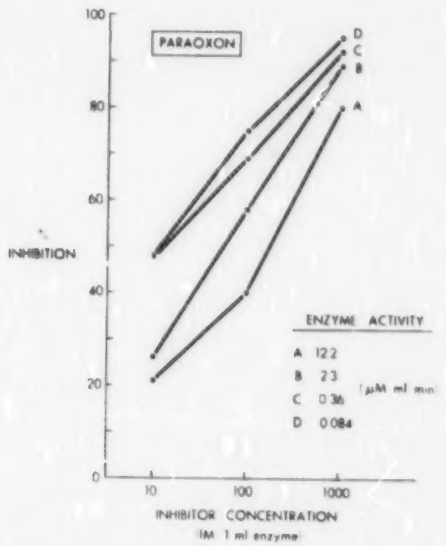


Figure 92. Per cent inhibition of four true cholinesterase activities by three concentrations of paraoxon.

The paraoxon concentrations used are expressed in femto moles (10^{-15}). This illustration demonstrates that for paraoxon the sensitivity of the assay is in the femto gram region.

Figure 93 illustrates the inhibition characteristics of malaoxon in the pico mole range on the various enzyme dilutions. There is roughly a 100 fold difference in the inhibitory effectiveness of paraoxon and malaoxon in this assay procedure. This difference roughly parallels the difference in the intravenously administered LD_{50} of the two compounds.

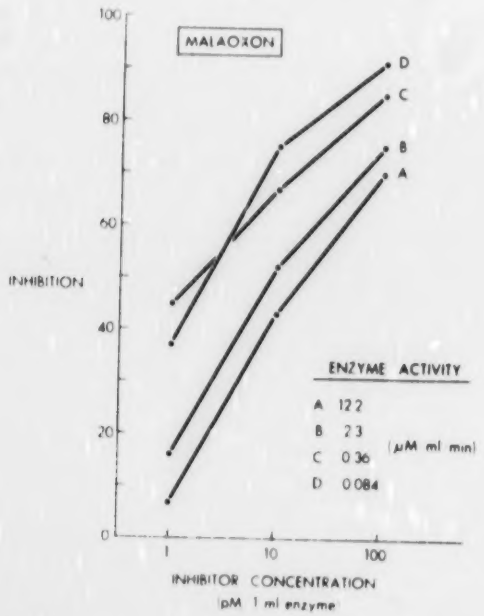


Figure 93. Per cent inhibition of four true cholinesterase activities by three concentrations of malaoxon.

Figure 94 illustrates the inhibition characteristics of carbaryl in the nano mole range. There is roughly a 100,000 fold difference in the inhibitory effectiveness of paraoxon and carbaryl and again this indicates the order of magnitude separating the toxicities of the two compounds.

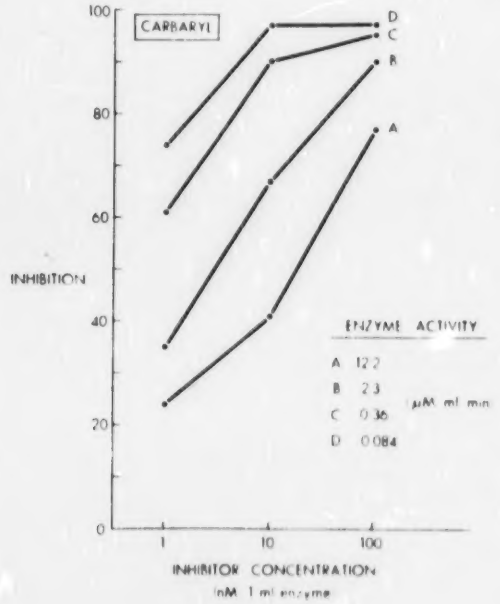


Figure 94. Per cent inhibition of four true cholinesterase activities by three concentrations of carbaryl.

Table 45 presents the means and standard errors of six replicate samples. In each case the inhibitor concentrations produced about 50% inhibition of the enzyme.

Table 45

Mean and Standard Error for Six Identical Samples

Enzyme Activity	*A*	*B*	*C*	*D*
Carbaryl				
Mean µM/ml/min	5.4	0.64	0.065	0.0046
S. E.	0.11	0.01	0.001	0.0004
Malaoxon				
Mean µM/ml/min	6.10	1.0	0.154	0.0235
S. E.	0.11	0.02	0.001	0.0005

Table 46 shows mean and standard error of day to day variation. The values are of seven determinations made on a given sample of enzyme in a two month period.

Table 46

Mean and Standard Error of Day to Day Variation of Seven Determinations

	A	*B*	*C*	*D*
Mean M/ml/min	11.2	2.2	0.41	0.083
S. E.	0.4	0.07	0.02	0.003

The data from which Table 47 was derived support the observation that the assay procedure is sufficiently precise to distinguish at the 95% confidence level the difference between *1* and *3* pico moles of malaoxon.

Table 47

Illustration of Inhibitor Concentration and % Inhibition

Enzyme Activity	*Sample*	*% Inhibition*	
µM/ml/min.	*Inhibitor conc. in moles*	*Expected**	*Observed†*
A	1 - $1x10^{-6}$	44	41
12.2	2 - $3x10^{-6}$	59	59
	3 - $5x10^{-5}$	66	66
B	1 - $1x10^{-6}$	51	48
2.3	2 - $3x10^{-6}$	64	67
	3 - $5x10^{-5}$	70	74
C	1 - $1x10^{-6}$	61	54
0.36	2 - $3x10^{-6}$	72	73
	3 - $5x10^{-5}$	77	82
D	1 - $1x10^{-6}$	70	62
0.084	2 - $3x10^{-6}$	81	80
	3 - $5x10^{-5}$	86	86

* Results expected from standard curve.

† Results obtained experimentally.

Samples of malathion, parathion, carbaryl and other anticholinesterase compounds have been analyzed in both ethylene glycol, a common solvent used in air samplers, and in potable water. Quantities of these compounds were determined both by standard analytical procedures and by the enzyme assay technigue, which produced results that were in agreement.

The anticholinesterase activities of samples containing direct inhibitors and components which must be activated can be divided by determining duplicate samples of which only one is treated with bromine water.

This assay technique shows much promise for further development in the area of environmental sample evaluation.

For most screening procedures, the oxidation of all samples and the use of a single, probably B, enzyme concentration would be sufficient to identify samples which might require further analytical evaluation.

SUMMARY

A procedure has been described which is applicable to the screening of large numbers of samples for anticholinesterase activity. The method is sufficiently sensitive to detect cholinesterase depression due to as little as 30 ppt paraoxon; 3 ppb malaoxon or 2 ppm of carbaryl in either ethylene glycol or water. While the method does not allow the absolute differentiation between compounds which might be present, some classification is possible, *i.e.*, for compounds which must be activated before they possess anticholinesterase activity. The technique can be made more specific through manipulations of types of cholinesterase used and substrates employed. The major usefulness of the technique at this time is for screening.

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PUBLICATION 266

PESTICIDES PROGRAM
National Communicable Disease Center
Atlanta, Ga. 30333

A Gas Chromatographic Method for the Determination of Low Concentrations of Abate in Water

by M. T. SHAFIK[1]

Abate (0,0,0',0'-tetramethyl-0,0'-thiodi-p-phenylene phosphorothioate) is a larvicide suggested for the control of mosquito larvae in natural waters.

Data are needed for Abate residues in water in order to determine the levels which will result from the routine use of this compound in drinking waters.

Abate is determined by its hydrolysis to 4,4'-thiodiphenol which in turn can be determined spectrophotometrically in the ultraviolet region or can be reacted with 4-aminoantipyrine to give a color to be used as the basis for a colorimetric procedure (1). Abate was also determined using a flame ionization detector and a DC-11 column maintained at 270°C (2). The colorimetric methods lack sensitivity, and the gas chromatographic method lacks both sensitivity and reproducibility, as Abate and its hydrolysis product 4,4'-thiodiphenol have a low vapor pressure which discourages the use of gas liquid chromatography.

The injection of a large number of Abate samples in the gas chromatograph contaminate the instrument with the result that Abate peaks appear with the solvent injection only.

The following investigation is concerned with the quantitative conversion of the low vapor pressure 4,4'-thiodiphenol to a product of high vapor pressure using silylating reagents.

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The replacement of the active hydrogens in the two hydroxyl groups with Si $(CH_3)_3$ groups enhance the passage of the compound through the gas chromatograph.

Materials and Methods

Reagents.

Trimethylchlorosilane - Analabs, Hamden, Conn.[2/]
Hexamethyldisilazane - Analabs, Hamden, Conn.
Chloroform, redistilled
n-Hexane, dry, redistilled
Methanol, redistilled
Acetone, redistilled
Aqueous potassium hydroxide solution 15N
Aqueous sodium hydroxide solution 0.1N
Sulfuric acid solution 1N and 6N
Sodium sulfate, anhydrous

Apparatus

Gas chromatograph model MT 220 equipped with a flame photometric detector, sulfur filter and a U-shaped aluminum column, ¼" o.d. x 4' packed with 80/100 mesh Chromosorb W coated with 2.5% E 301 and 0.25% Epon 1001 (w/w). The temperatures are; inlet 190°C, column 190°C, detector 165°C. The flow rates are, nitrogen 100, hydrogen 150, oxygen 15 ml./minute. On column injection was used in this work.

Analytical Procedure

Preparation of standard curve. Dissolve 0.1000 gram of the 4,4'-thiodiphenol standard in one liter of dry benzene. Pipet 0.02, 0.04, 0.06 and 0.08 ml of the standard solution in 25- ml graduated concentrator tubes

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2/ Commercial sources and trade names[2] are provided for identification only. Their mention does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education and Welfare.

corresponding to 2,4,6 and 8 micrograms respectively. Add dry benzene to the 1-ml mark. To the 4,4'-thiodiphenol add 0.25 ml dry pyridine, 0.15 ml hexamethyldisilazane, 0.05 ml trimethychlorosilane; stopper, mix and heat at 50°C for 10 minutes with caution.* Allow tubes to stand overnight at room temperature. Add approximately 5 ml water then 5 ml 1N sulfuric acid to make the solution acidic and shake vigorously. After separation of the two layers, aspirate off the water layer. Repeat the water washing of the n-hexane or benzene layer two more times. Adjust the volume of the remaining n-hexane or benzene so that 10 microliters will contain 2-20 or 20-80 nanograms 4,4'-thiodiphenol. Plot the response or peak height versus concentration on regular or log log graph paper.

Extraction. Filter a 500 ml sample of water into a liter separatory funnel. Rinse the filter and container with two 15 ml portions of acetone and add to separatory funnel. Add 100 ml chloroform via the filter. Shake the funnel vigorously for one minute. Transfer the chloroform layer to a second separatory funnel. Wash the chloroform with 25 ml of 0.1N sodium hydroxide solution. Dry the chloroform extract by passing through a column of anhydrous sodium sulfate into a Kuderna-Danish concentrator fitted with a 15-ml round bottom test tube. Repeat the extraction, washing and drying steps with two additional portions of fresh chloroform and combine the extracts in the Kuderna-Danish concentrator. Concentrate the extract on a steam bath, then evaporate the last trace of chloroform using a gentle stream of dry nitrogen, and save residue for the hydrolysis step.

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*It is necessary to handle the silylation reagents in a well-ventillated hood.

Hydrolysis. Dissolve the Abate residue from a standard or from a water extract in 4 ml methanol. Add 1 ml 15N potassium hydroxide solution. Place a one-ball Snyder column in the top of the tube and heat at 100°C until all the methanol has evaporated. Remove the Snyder column and continue to heat at 100°C for one hour. Cool and dissolve the residue in 5 ml of water; then transfer the hydrolysate to a 50-ml separatory funnel with the aid of 10 ml of chloroform. Acidify the aqueous layer with 5 ml 6N sulfuric acid and extract with a total of two 20-ml portions and one 10-ml portion of chloroform. Dry the combined chloroform extracts by passing through anhydrous sodium sulfate into a Kuderna-Danish evaporator fitted with a 25-ml evaporative concentrator tube. Concentrate the extract to a volume of about 5 ml on a steam bath. Evaporate the last 5 ml of chloroform with the aid of a gentle stream of dry nitrogen and a room temperature water bath.

Dissolve the residue in 1 ml of dry n-hexane or dry benzene. Stopper and heat the tubes at 50°C for about 10 minutes to dissolve the hydrolysate "4,4-thiodiphenol."

Silylation reaction. Add to the dissolved Abate hydrolysate, 0.25 ml dry pyridine, 0.15 ml hexamethyldisilazane, 0.05 ml trimethylchlorosilane; stopper, mix and heat at 50°C for 10 minutes with caution. Allow tubes to stand overnight at room temperature. Add approximately 5 ml water then 5 ml 1N sulfuric acid to make the solution acidic and shake vigorously. After separation of the two layers, aspirate off the water layer. Repeat the water washing of the n-hexane or benzene layer two more times. Adjust the volume of the

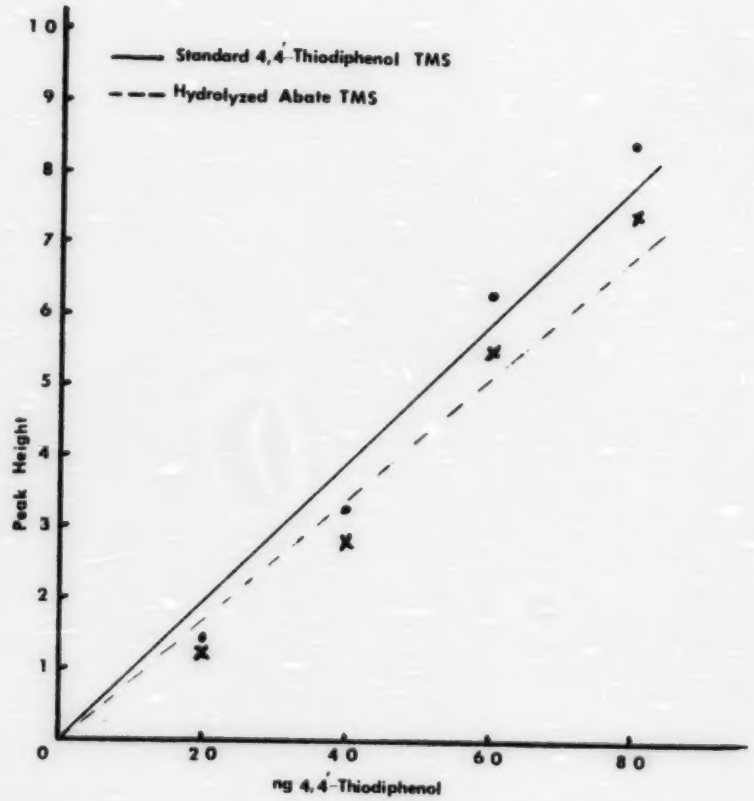


Fig. 1 Standard curves of 4,4'-thiodiphenol and hydrolyzed abate TMS

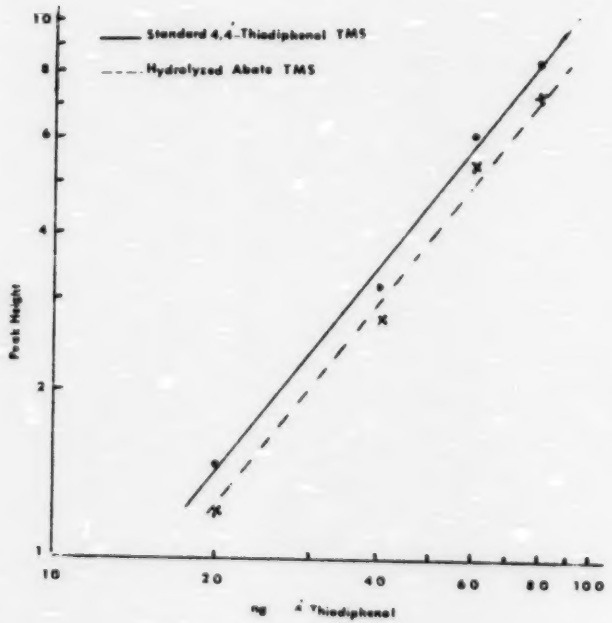


Fig. 2. Standard curves of 4,4'-thiodiphenol TMS and hydrolyzed abate TMS

remaining n-hexane or benzene so that 10 microliters will contain 2-20 or 20-80 nanograms 4,4'-thiodiphenol.

Results and Discussion

Four, 500-ml water samples were fortified with 2,3, 6, 16 micrograms of Abate. Each sample was carried through the entire analytical procedure. Standards of 4,4'-thiodiphenol were treated with the silylation reagents and analyzed by gas chromatography in order to determine the recovery of Abate from the fortified water samples. The results are shown graphically in Figures 1 and 2. The average recovery of Abate from the fortified water samples, when compared with the 4,4'-thiodiphenol standards, was 88%.

The hydrolysis of Abate with 15N potassium hydroxide using the procedure suggested by Blinn and Pasarela (1), is essentially complete. When aliquots of the hydrolysate were spotted, developed and detected on a silica gel fluorescein treated plate, using a 1:1 mixture of n-hexane and diethylether and an ultraviolet lamp, the R_f value was identical with that of pure 4,4'-thiodiphenol.

The chromatogram in Figure 3 shows the response of the flame photometric detector equipped with the sulfur filter to the hydrolysis product of Abate, 4,4'-thiodiphenol. Higher attenuations can be used depending on the noise level of the detector.

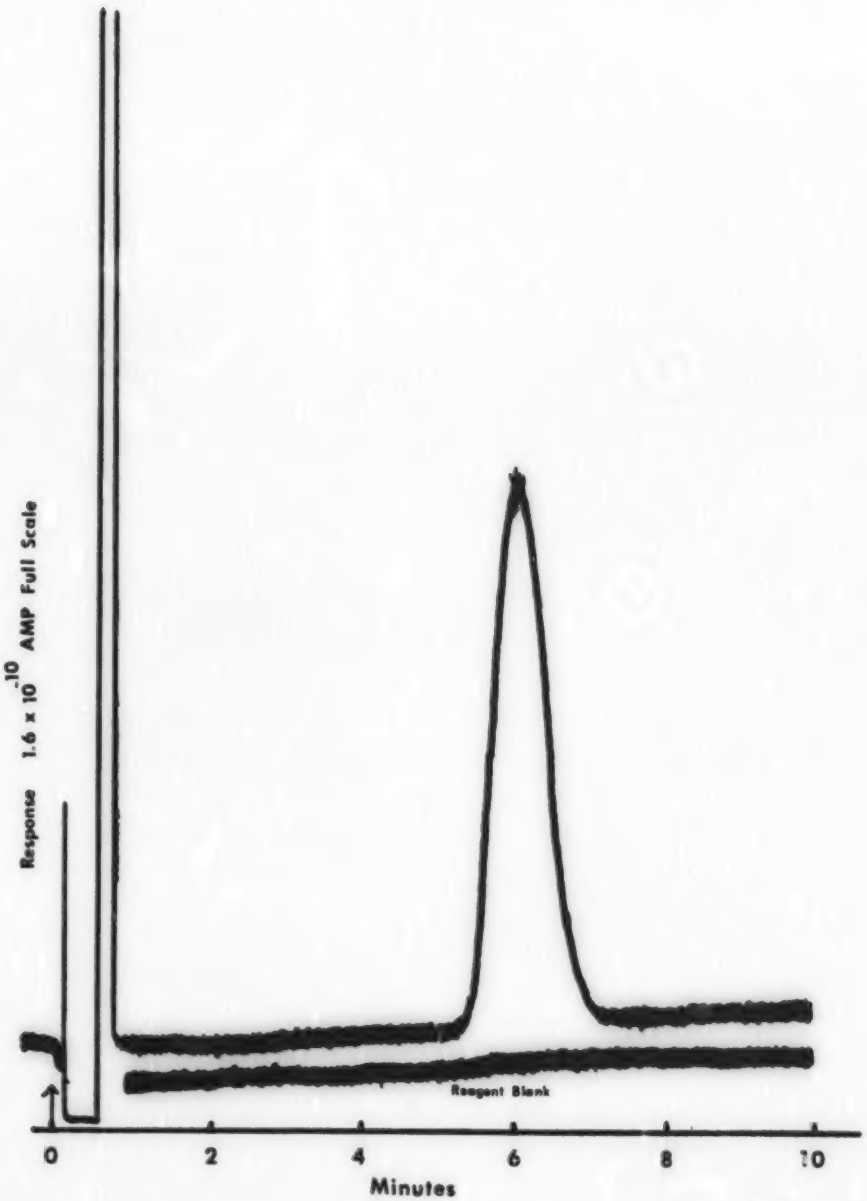
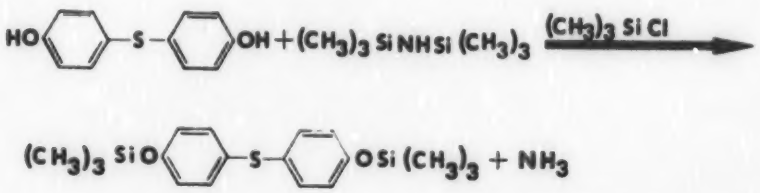


Fig. 3. Chromatogram of 10 ng of 4,4'-thiodiphenol TMS

The replacement of the active hydrogens in 4,4'-thiodiphenol with TMS groups can be represented by the following reaction:



The trimethylchlorosilane "TMCS" serves to catalyze the reaction and the hexamethyldisilazane "HMDS" is the silylating reagent.

It was not possible to inject this reaction mixture directly in the gas chromatograph, as the silylating reagents deposited on the windows on the detector. Such deposits decrease the intensity of the light transmitted to the photomultiplier tube which in turn lowers the sensitivity. In order to avoid this problem it is necessary to remove the excess reagents by acidifying and washing with water several times.

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Gas Chromatographic Determination of Pentachlorophenol in Human Blood and Urine

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PESTICIDE PUBLICATION
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Environmental Protection Agency
Chamblee, Georgia 30341

The Hawaii Community Studies on Pesticides has used methods developed by BEVENUE et al. (1966,1968) to determine pentachlorophenol(PCP) residues in human blood and urine. The PCP-blood method has also been applied to the determination of 2,4-D and dicamba in human blood and urine (RIVERS et al. 1970).

The modified PCP analytical procedure described in this paper represents an efficient combination of features taken from the procedures previously used, plus the use of a mechanical mixing technique developed by THOMPSON (1970) for extracting organochlorine pesticides from human serum samples. The procedure retains the accuracy of the procedures previously used, requires less time and equipment to perform, and applies to both human blood and urine.

Experimental

Apparatus. Extractions were carried out in 16X125 mm culture tubes fitted with screw caps with teflon-lined rubber liners. Samples were mixed on a Roto-Rack™, a rotary mixing device available from Fisher Scientific Company.

Gas chromatographic determinations were made on a MicroTek MT 220 gas chromatograph under conditions previously described(RIVERS et al. 1970).

Extraction and Methylation. Two ml of human blood plasma or urine were combined with 6.0 ml of benzene and 2 drops of conc. H_2SO_4 in a culture tube. The tube was sealed and rotated on the "Roto-Rack" for two hours at 50 r.p.m. After mixing, the sample was centrifuged briefly to bring about phase separation, and 3.0 ml (representing 1.0 ml of sample) of the benzene (top) layer were transferred to a 10-ml volumetric flask. Diazomethane solution (e.2 ml), prepared as previously described (RIVERS et al. 1970), was added to the extract, and the mixture was allowed to stand for 15 min. Excess diazomethane was removed by gently bubbling dry nitrogen through the extract until the yellow color disappeared, and the extract was diluted to 10.0 ml with isooctane.

Gas Chromatography. The methylated extract was diluted as necessary, and 2 to 8 ul were injected into the gas chromatograph. Following each extract injection, an amount of PCP standard (as

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its methyl ether) which gave a response of similar peak height (±10%) was injected, and results were calculated by direct comparison of peak height.

Results and Discussion

Results of a recovery study to determine the accuracy and reproducibility of the method over the range of PCP levels encountered in samples in this laboratory are presented in Table 1.

TABLE 1

Recovery of PCP from samples fortified before extraction.

Sample	PCP found (p.p.m.)	PCP added (p.p.m.)	Average[a] (p.p.m.)	Recovery (%)	Std. Dev. (%)
Blood Plasma	0.19	0.10	0.28	90	±3.1
		0.50	0.67	96	±3.2
		5.00	4.66	89	±1.2
		50.0	45.8	91	±6.7
Urine	0.01	0.10	0.10	90	±2.7
		0.50	0.48	94	±4.0
		5.00	4.82	96	±2.1
		50.0	49.5	99	±1.2

[a]Average of three values.

PCP levels in pooled blood plasma and urine samples were determined by the methods of BEVENUE et al.(1966,1968). Aliquots of the samples were then fortified with PCP at various levels (see Table 1), and each fortified aliquot was analyzed in triplicate. Recoveries of PCP ranged from 89% to 96% for blood plasma and from 90% to 99% for urine. Standard deviation from the mean value was less than ±5% for each type of sample, and the limit of detectability for the method is 0.01 p.p.m.

A comparison of PCP levels found in routine blood samples by the method of BEVENUE et al.(1968) and by the method detailed in this paper is given in Table 2. As expected, the results obtained

TABLE 2

Comparison of PCP levels found in routine blood plasma samples by two methods.

Sample	PCP found (p.p.m.)	
	Method of BEVENUE et al.(1968)	Present Method
B1	47.3	45.4
B2	5.18	5.32
B3	0.65	0.68
B4	0.17	0.19
B5	0.12	0.14
B6	0.08	0.07
B7	0.07	0.07

by these methods are in good agreement since the accuracy and reproducibility of the two methods are essentially similar. It follows that past data obtained using the old procedures can be compared directly with data obtained using the new procedure without the need to apply a derived factor to make the comparison valid.

No attempt was made to confirm the identity of PCP as detected by this procedure. The presence of PCP in blood and urine samples obtained from residents of Hawaii has long been established and need not be re-investigated. For investigators beginning PCP analyses, the confirmation can be done using thin layer chromatography and IR spectrophotography as reported by BEVENUE et al.(1966,1968).

No interferring responses were encountered in any of the samples analyzed. However, as previously reported (RIVERS et al. 1970), the use of the diazomethane solution in amounts greater than those indicated in the procedure can lead to interferences due to impurities in the reagent.

The real contribution of this procedure to the author's laboratory, beyond its accuracy and precision, is the significant decrease in time and equipment required to perform each analysis. Time-consuming transfers, centrifugations and concentration steps have been eliminated along with the equipment needs involved. The rate at which samples can be analyzed is now limited only by the time required for the GLC analysis.

Acknowledgements

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PUBLICATION

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Construction of a Leak-Inlet System for the LKB 9000 Gas Chromatograph–Mass Spectrometer*

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(Received 26 February 1970; revision received 15 May 1970)

INDEX HEADINGS: Gas chromatograph–mass spectrometer; Leak-inlet system.

Apart from the direct inlet system which permits the introduction of substances with very low volatility and/or thermal stability into the mass spectrometer, the LKB instrument also permits the high speed recording of mass spectra of volatile compounds as single components or components of mixtures eluted from a gas chromatograph. Caution must be exercised in the interpretation of these spectra in view of possible peak or component overlap using the gas chromatographic column. At times it is useful to have another alternative system for the rapid introduction of controlled amounts of gaseous substances and/or highly volatile liquids and solids as well as reference samples such as the perfluoroalkanes directly into the ion source of the mass spectrometer for analysis. A heated molecular leak system for this purpose was developed (see Fig. 1). An inexpensive leak-inlet system which is satisfactory for the highly volatile and reference substances can be easily constructed, and thereby greatly increases the versatility of the instrument. The sample introduction tube is an 8 mm ($\frac{5}{16}$ in.) o.d. stainless steel tube inserted into the analyzer tube in the same way as a direct probe. The tube is flared at one end and attached to a Manostat flow controller, 78-425-01, or similar valve. An inner Teflon tube, $\frac{3}{16}$ in. o.d.×1 mm i.d., [illegible] Teflon ferrule in the flared end of the steel tube [illegible] a chemically inert pathway and the flow restriction necessary for sensitive control at low pressures. A standard 8 mm o.d. Manostat glass joint into which has been inserted a silicone rubber plug septum (the same as that used by LKB's GC column) comprises the sample reservoir. For samples of low volatility, the glass inlet can be heated by an aluminum block containing a cartridge heater (Fig. 2).

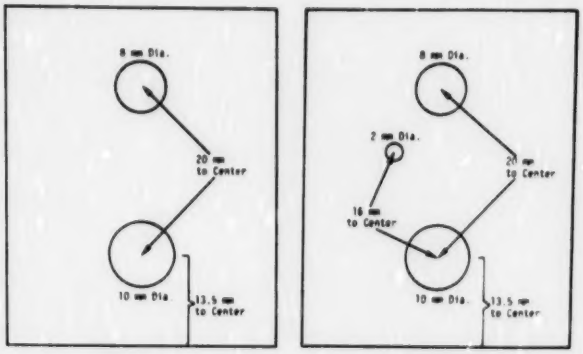

FIG. 2. Sample chamber heater block (aluminum, 45×40×50 mm). Parts bored in the 40×50 mm face.

This system allows the introduction of a constant amount of sample through the flow controller for periods ranging from several seconds to over a minute, depending on the sample size and desired concentration in the analyzer tube. We have found this simple system to be very useful for the analysis of trace contaminants of gases by mass spectroscopy.

* The use of trade names is for identification purposes only, and does not constitute endorsement by the Food and Drug Administration or the Department of Health, Education, and Welfare.

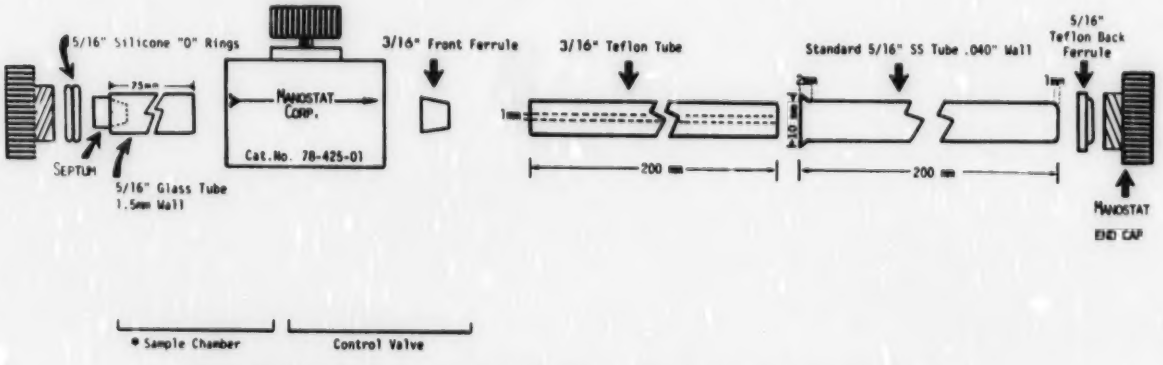

FIG. 1. Leak-inlet system. *Standard Manostat tubing can be used on sample chamber.

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PUBLICATION

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TOXICOLOGY PROGRAM
Technology Branch
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INSECTICIDE DETERMINATION

Colorimetric Determination of Dieldrin and Its Application to Animal Fat

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This method for determining microgram quantities of dieldrin is based on the color complex formed between dieldrin and diphenylamine in the presence of zinc chloride. The absorption at 650 mμ by color reaction products in an acetic acid solution follows Beer's law. The method is general for most chlorinated insecticides, but specificity is gained through alkaline hydrolysis and chromatography.

THE EXTENSIVE USE of insecticide commonly known as dieldrin or HEOD (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) has created the need for a simple, sensitive method of analysis adaptable to routine determinations. Such a method is of prime importance in the study of the toxicity and hazards of dieldrin to humans, and might also find use in the determination of residues on food products and in the analysis of formulations.

An evaluation was made of the existing methods reported in technical literature for the colorimetric determination of microgram quantities of dieldrin. Bioassay and total organic chlorine techniques were not considered because of the lack of specificity. The only thoroughly described colorimetric procedure is that of O'Donnell, Johnson, and Weiss (*9*), which is long and tedious and subject to unduly high and variable blank values.

Davidow and Laug (*2*) reported a method involving the reaction of dieldrin with anhydrous hydrobromide in dioxane. The method is rather long, and the requirement of anhydrous hydrobromide limits its convenient use as a routine method.

Gunther, Kolbezen, and Blinn (*5*), and more recently Johnson (*6*), have reported on color reactions for the detection of dieldrin. These techniques have not as yet been expanded into quantitative methods of analysis. The color reaction of Gunther *et al.* appears to give inconsistent results, and that of Johnson lacks the desired sensitivity.

In 1945, Jones (*7*) and Lemley (*8*) reported on a color reaction of DDT with zinc chloride, phenol, and diphenylamine. Graupner has developed a method for toxaphene (*4*) based on the blue-green color reaction of this insecticide with diphenylamine and zinc chloride. On investigating the possibility that this color reaction under certain conditions is a more general one (*3*), it was noted that dieldrin gave a purple color of an intensity suitable for colorimetric determination.

The method described in this paper involves saponification, extraction, chromatography, and color development. In a final volume of 1.0 ml. of solution and using a semimicro absorption cell having a 10-mm. light path, a sensitivity of 5 γ with about 7% variation can be obtained. The method has been applied to the determination of dieldrin added to animal fat. Although all the possible variations have not been studied, it is believed that the following description will satisfy the immediate need for a sensitive, simple, routine method of determining dieldrin and will facilitate the expansion of the method to other biological materials and to metabolic and residue studies.

Apparatus

Beckman spectrophotometer, Model B, with red-sensitive phototube.

Aluminum heating block containing holes 22.5 mm. in diameter and 50 mm. deep. The block should be maintained at 205° ± 5° C.

Glass gas-drying unit charged with indicating Drierite.

Reagents

Diethyl ether, reagent grade. Ether containing peroxides is unsatisfactory and should be purified and redistilled before using. To test for peroxides, shake a small portion of the ether with an aqueous potassium iodide solution. The release of free iodine, as determined by the starch test, indicates the presence of peroxides. These are decomposed by washing the ether with a ferrous sulfate or sodium bisulfite solution until the potassium iodide test is negative. The ether is then washed with water, dried with sodium sulfate, and redistilled.

Alumina, adsorption grade, 80 to 200 mesh. Do not activate.

Diphenylamine solution, 0.25%. Dissolve 250 mg. of diphenylamine, recrystallized at least twice from hexane, in 100 ml. of redistilled *n*-hexane.

Zinc chloride reagent, 0.25%. Dissolve 250 mg. of zinc chloride in purified, redistilled ether. A slight suspension is formed which precipitates on standing. Resuspend before use. Do not filter through paper. This reagent is unstable and must be prepared each day.

Solvent for colored reaction products, 10% by volume of C.P. acetic anhydride in C.P. glacial acetic acid.

Dieldrin standard solutions. Dieldrin (at least 99.5% pure) is dissolved in purified redistilled *n*-hexane to give solutions containing 1, 10, and 20 γ per ml.

Procedure

Preparation of Standard Curves. Standard curves are prepared in three ranges of concentrations, depending upon the required sensitivity. In the 1- to 10-γ range, the colored products are dissolved in 1.0 ml. of solvent, and the absorbance is measured in a microabsorption cell having a 50-mm. light path. In the 5- to 50-γ range, the colored products are dissolved in 1.0 ml. of solvent, and a semimicrocell is used having an 0.8-ml. capacity and a light path of 10 mm. At the higher concentrations, 20 to 150 γ, a total volume of 3.0 ml. of solvent is used. The absorbance is measured in cells having a 10-mm. light path and a 3-ml. capacity.

Appropriate aliquots of the standard solution of dieldrin to give at least four points on each curve are pipetted into test tubes (22 × 175 mm.). The samples are evaporated just to dryness in a water bath at 40° C. with the aid of a

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gentle stream of dry, clean air filtered through a laboratory glass gas-drying unit. Two milliliters of the diphenylamine and zinc chloride reagents are added and the samples are again evaporated to complete dryness under the same conditions as before. A colorless, dry residual film should appear in each tube. The samples then are placed in the aluminum heating block at 205° ± 5° C. for exactly 3.0 minutes. The tubes are removed and cooled in running tap water. The colored reaction products are dissolved in either 1.0 or 3.0 ml. of the 10% acetic anhydride in glacial acetic acid solvent, depending upon the range of the curve being studied. The absorbance of the samples is read at 650 mμ within 10 minutes after the addition of the solvent; water or the acid solvent itself is used as reference. A reagent blank is carried through with the standards under exactly the same conditions. Plotting micrograms of dieldrin *vs.* absorbance on rectangular coordinate paper should give straight-line curves.

Analysis of Animal Fat. SAPONIFICATION AND EXTRACTION. To a weighed amount of animal fat (1.0 gram) in a 300-ml. ₹ ground-glass-joint Erlenmeyer flask, 15 ml. of 95% ethanol (redistilled) and 3 ml. of 50% aqueous potassium hydroxide are added. The sample is refluxed gently for at least 1 hour or until a homogeneous solution is obtained. It is then removed from the source of heat and cooled, and 10 ml. of water and 50 ml. of redistilled *n*-hexane are added. The sample is shaken vigorously and the phases are allowed to separate. This operation is repeated three times, and then the lower phase is aspirated off. The organic solvent extract is washed once with 50 ml. of 1*N* hydrochloric acid, once with 25 ml. of water, and once with 25 ml. of 20% sodium chloride, the aqueous layer being aspirated off each time. The extract then is dried with sodium sulfate and a 40-ml. aliquot pipetted into a test tube. The sample is evaporated with a gentle stream of air in the 40° C. water bath to a volume of about 5 ml.

CHROMATOGRAPHY. The chromatographic columns are prepared by adding small amounts of dry adsorption alumina and tapping the tubes (10 × 33 mm.) gently to assure packing. This is repeated until a column of adsorbent 10 cm. in height is obtained. A plug of glass wool confines the alumina.

The *n*-hexane extract of the saponified fat is transferred to the column with the aid of a few milliliters of the 10% diethyl ether in hexane eluent. When the extract is completely taken up into the alumina, the column is eluted with 10% ether in hexane, and three 40-ml. samples of the eluate are collected in test tubes (22 × 175 mm.). The samples are evaporated just to dryness in a water bath at 40° C. with the aid of a stream of dry clean air. Two milliliters each of the diphenylamine and zinc chloride reagents are added, and the analysis is completed as for the preparation of the standard curves. A column blank is run through both the chromatographic and color reaction procedures. Its absorbance at 650 mμ is used as a correction for the absorbance value of the fat sample.

In the determination of unknown quantities of dieldrin, the amount of the colored reaction product formed after fusion offers a visual aid in determining whether this residue should be dissolved in 1.0 or 3.0 ml. of acetic acid solvent. This then establishes the absorption cells and the standard curve to be used. Alternatively the fusion products are dissolved in 1.0 ml. of the acid solvent. Then, if the color intensity of this solution warrants it, further appropriate dilutions are made.

It is important to run a blank analysis through the entire procedure on a sample of the same amount and type of material as that being analyzed. Further, a sample of the standard solution of dieldrin should be run through the chromatographic procedure to determine the quantitative recovery of dieldrin in the three eluates.

Results and Discussion

Standard Curves. Acetone, methanol, and ether were used as solvents for the two color reagents, diphenylamine and zinc chloride. The three solvents appeared to be satisfactory, but further work revealed that acetone and methanol gave higher reagent blank values than ether. This difference became significant when micro absorption cells were used. Care must be taken that the ether is pure and does not contain peroxides. These oxidation products cause high blank values and, depending upon their concentrations, may even cause extraneous color in the fused residue.

In the early work on this method, evaporation of the ether solution of the two color reagents, prior to fusion, occasionally gave a residue containing visible amounts of moisture. Furthermore, if a few milliliters of *n*-hexane were mixed with the ether solutions, the evaporation proceeded satisfactorily to complete dryness, and a colorless residual film was obtained at the bottom of the test tube. This same satisfactory effect was consistently achieved by preparing the diphenylamine reagent in *n*-hexane. The reagent blank values were not altered.

Varying the concentration of the color reagents between 0.25 and 1.0% did not increase the sensitivity of the method but increased the blank values. The effect of varying the reaction temperature on the absorbance of the color developed by dieldrin is shown in Table I. Reaction time was maintained constant at 3.0 minutes. The data show an increase in color intensity and in blank values with an increase in temperature. Varying the reaction time from 1 to 10 minutes and maintaining the temperature at 205° C. resulted in essentially the same effect—increased blank values and color intensity with some decomposition at the maximum time. These results indicate that the optimum time and temperature for the reaction of dieldrin with diphenylamine are the same as those reported for toxaphene (*4*)—e.g., 3 minutes at 205° C.

Table I. Effect of Reaction Temperature on Intensity of Color Developed by Dieldrin with Reference to Water

(Time of reaction, 3.0 minutes. Volume of colored solutions, 3.0 ml.)

Temp., ° C.	Absorbance at 650 Mμ: Reagent blanks	Absorbance at 650 Mμ: Dieldrin, 50 γ
125	0.003	0.007
160	0.014	0.124
205	0.020	0.337
225	0.093	0.420
250	0.170	Decomposed

Table II. Effect of Reaction Product Solvent on Intensity of Color Developed by 100 γ of Dieldrin

Solvent	Absorbance at 650 Mμ
Acetone	0.304
Methyl ethyl ketone	0.565
Methanol	0.158
Ethanol, 95%	0.160
2-Propanol	0.168
Ethyl acetate	0.200
Benzene	Insoluble
Glacial acetic acid	0.520
Acetic anhydride–acetic acid mixtures	
1:10	0.624
2:10	0.638
5:10	0.616

The effect of various solvents on the color intensity and thus the sensitivity of the method was studied using a reaction time of 3 minutes at 205° C. The representative data given in Table II indicate that, of the solvents studied, the acetic anhydride–acetic acid mixtures permitted the maximum development of color.

The color produced in all of the solvents studied faded rather rapidly. In the acetic anhydride–acetic acid solvent, the loss of color intensity is 15 to 20% in the first hour. It is therefore of prime importance that the absorbance of the samples be determined within a specified time. When the absorbance was measured at random, but within 10 minutes of the addition of the acid solvent, the variation between several standard curves prepared was not greater

Table III. Variables in Standardization of Colorimetric Determination of Dieldrin in Three Concentration Ranges

Variables	Range, γ 1–10	5–50	20–150
Volume of colored solution, ml.	1.0	1.0	3.0
Light path of cells, mm.	50	10	10
Maximum absorption, mμ	650	650	650
Reagent blank at λ_{max} abs.	0.100	0.020	0.010
Absorptivity	0.0869	0.0191	0.00641
Variation, %	7	7	5
Sensitivity, γ	1.0	5.0	15

Table IV. Recovery from Rabbit Fat of Dieldrin Added at Various Concentrations

Dieldrin, P.P.M. Added	Found	Recovery, %
20	18	90.0
40	39	97.5
80	75	93.8
100	94	94.0

Table V. Recovery of 100 γ of Dieldrin Added to 1 Gram of Animal Fat

Source of Fat	Dieldrin per Eluate, 40-Ml. Fractions, % 1	2	3	Total Recovery, %
Human A[a]	75	16	0	91
Human B[b]	68	24	0	92
Monkey	90	0	0	90
Rabbit	91	0	0	91
Rat	83	14	0	97
Dieldrin[c]	55	37	0	92

[a] Obtained in 1958.
[b] Obtained before advent of chlorinated insecticides.
[c] Standard dieldrin, no fat, subjected to complete analysis.

than 7%. This is satisfactory for most colorimetric determinations. Representative data pertinent to the standardization of the method are given in Table III.

Analysis of Animal Fat. SAPONIFICATION AND EXTRACTION. Davidow (*1*) has described a convenient technique for the isolation of the insecticide DDT from fat using a sulfuric acid–impregnated Celite column. However, this effective cleanup method is not applicable to dieldrin because the compound, unlike DDT, is not stable to the action of concentrated mineral acids but is stable to that of alkali. The latter property was utilized by O'Donnell *et al.* (*9*) in the isolation of dieldrin from glycerides.

In the present study, 1-gram samples of rat, rabbit, monkey, and human fat were saponified directly. Analysis of materials other than fat may require extraction prior to saponification. During the initial extraction of the saponified mixture, emulsion formation is avoided by adding only a minimum amount of water. No emulsions were formed in any of the samples analyzed when the alcohol content was reduced to 50 to 60% by the addition of water.

In the evaporation procedures, if a stream of air is used to aid in the complete removal of the solvent, the samples must be maintained at or below 40° C. to prevent loss of insecticide. If air is not used, hexane distillation temperatures may be utilized to concentrate extracts or eluates without loss of dieldrin. In any event, the evaporation should never be prolonged.

CHROMATOGRAPHY. O'Donnell *et al.* (*9*) described a magnesium oxide–Celite chromatographic column used in the isolation of dieldrin from extracts of saponified glycerides. This column is effective but requires vacuum or pressure to produce a flow of the eluents and necessitates collection of large volumes of eluate. A less time-consuming technique was desired.

After several adsorbents had been evaluated, a simple chromatographic procedure was developed capable of isolating dieldrin from an extract of saponified fat. Columns 10 mm. in diameter and packed 10 cm. high with adsorption grade alumina retained the impurities of an extract of 1 gram of saponified animal fat and permitted the quantitative recovery of dieldrin in about 80 ml. of eluate. A flow rate of 2 ml. per minute was used. If larger or different types of samples are to be analyzed, the column should be calibrated for its efficiency in cleanup. Column blank absorbance values were 0.015 to 0.030, using an absorption cell of 10-mm. light path and a 3.0-ml. final solution volume. Under the same conditions, control fat samples in general gave absorbance values of 0.020 to 0.050.

Table IV shows the recovery of known amounts of dieldrin added to rabbit fat. The eluates were collected in three 40-ml. fractions and analyzed individually and the results were totaled.

The recovery of 100 γ of dieldrin from 1 gram of different types of fat is shown in Table V. A fourth fraction of eluate, not shown in the table, was also collected and subjected to the dieldrin analysis. Although the added insecticide was recovered quantitatively in the first two fractions, the fourth fraction of eluates of some fat samples gave an extraneous violet color which faded rapidly. This was characteristic also of the human B fat sample, which was obtained from a patient before the advent of chlorinated

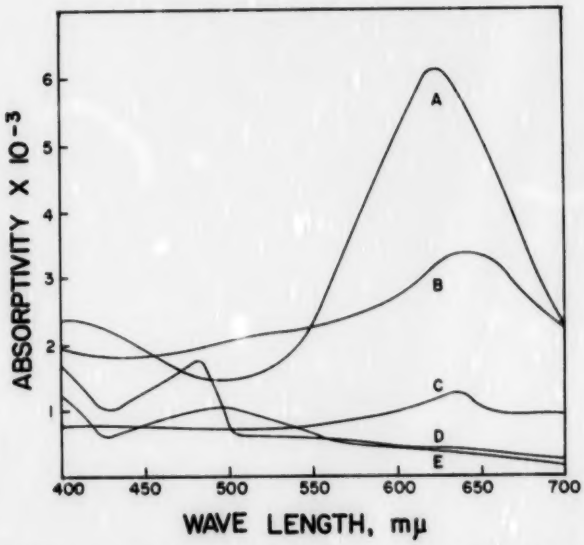

Figure 1. Spectra of chlorinated insecticides subjected to direct dieldrin colorimetric analysis

A. p,p'-DDD
B. Chlordan
C. Heptachlor
D. p,p'-DDT
E. o,p'-DDT

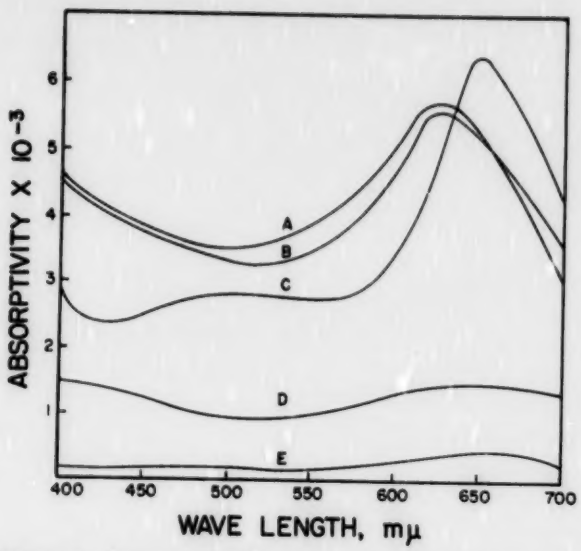

Figure 2. Spectra of chlorinated insecticides subjected to direct dieldrin colorimetric analysis

A. Strobane
B. Toxaphene
C. Dieldrin
D. Endrin
E. Aldrin

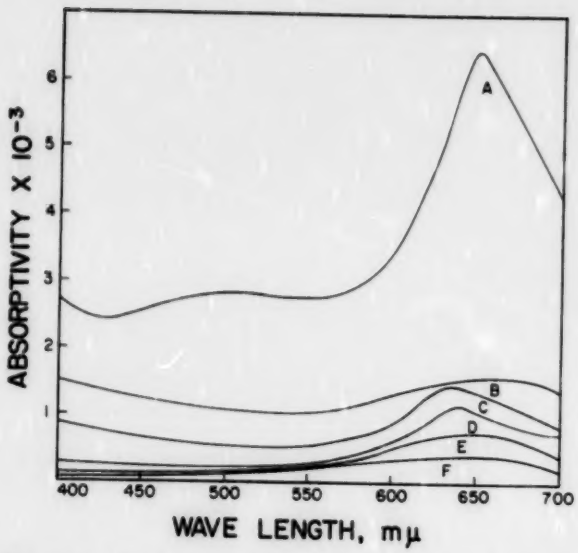

Figure 3. Spectra of chlorinated insecticides subjected to dieldrin colorimetric analysis after saponification and chromatography

A. Dieldrin
B. Endrin
C. Toxaphene
D. Strobane
E. Chlordan
F. Heptachlor

hydrocarbon insecticides. The findings indicate that continued elution of the chromatographic columns may result in recovery of a material of noninsecticidal origin. Therefore, adherence to the collection of eluates in fractions is desirable.

Reaction and Interference of Related Compounds. The nature of the color reaction is not thoroughly understood. Feigl (*3*) has reported that amines when melted with fluorescein chloride and anhydrous zinc chloride under conditions similar to those of this method produce a color complex. Fluorescein chloride is dehalogenated and the amine adds to the molecule. A similar reaction may be the basis of the colorimetric determination of dieldrin.

To determine the extent of this color reaction with related compounds, several chlorinated hydrocarbon insecticides were subjected directly to analysis. No saponification or chromatography was used.

Figures 1 and 2 give the spectra of the color complex formed with several insecticides and show that the wave length of maximum absorption and the intensity of color vary with most of the compounds. This may be due in part to variation of optimum conditions of color development for each material. For example, chlordan, when subjected to this analysis, gives an intense color without heating. Strobane also appears to be more reactive than toxaphene. DDE, DDA, dehydrochlorinated DDD, and lindane, not shown in the figures, gave no color reaction. From the spectra of some of the compounds certain possibilities are evident, such as the development of a simple method for the determination of DDD and its differentiation from DDT. This differentiation is not possible by the usual Schechter-Haller (*10*) procedure.

The general aspects of the reaction pose the problem of interference of related compounds on the determination of dieldrin. Some specificity is gained in the variations of color intensity and wave length of maximum absorption. To determine if further specificity could be gained through alkaline hydrolysis and chromatography, compounds shown in Figures 1 and 2 to offer maximum interference were subjected to the complete procedure described for the isolation of dieldrin from fat. Figure 3 shows both the spectra and the extent of interference of these insecticides after hydrolysis and chromatography. DDD on saponification is dehydrohalogenated to its ethylene derivative, which gives no characteristic color reaction. The instability of all of the compounds, except endrin, to alkaline hydrolysis is shown to be a means of limiting or of eliminating, by more intense saponification, their interferences in the dieldrin determination.

A confirmatory test for dieldrin was obtained by adding 0.2 ml. of concentrated sulfuric acid to the final colored solution. A characteristic color change occurred with the solution now having a maximum absorption at 540 mμ. The color is relatively stable but of low intensity. Oxidizing agents interfere with the test.

Acknowledgment

The author expresses appreciation to Alda J. Graupner for information on the colorimetric determination of toxaphene, which led to the development of this method for dieldrin.

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A Detector By-Pass Valve

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PUBLICATION
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PESTICIDES PROGRAM
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Gas chromatographic detection systems are subject to two main sources of interference: solvent elution and liquid phase bleed. Solvent elution, especially with large injection volumes, often produces undesirable tailing, over-loading or fouling of detector, and flame extinguishment. Column liquid phase bleed causes detector and combustion tube contamination which may result in lost sensitivity, anomalous behavior and catalyst poisoning. Most systems have attempted to circumvent these undesirable features by venting the solvent and discontinuing carrier flow when the detector is not in actual use. Solvent venting, while desirable, is not, as presently implemented, an entirely satisfactory answer, since discontinuing the carrier flow causes disruption of detector equilibrium or, in the case of flame detectors, discontinues ignition. In both instances, a re-equilibration period is required before detection equilibrium and response is restored.

The described problems would be solved by a system which would vent solvent, shunt column bleed, and maintain continuous detector equilibrium. Hardware for such a system is available commercially in the form of linear multiport* and micro volume switching** valves. Both valves operated satisfactorily up to a temperature and pressure of 220°C and 15 psia. The system is described utilizing the linear multiport valve, accepting ⅛ in Swagelok tube fittings, installed in the detector compartment of a Micro-Tek MT-220 gas chromatograph. These valves have also been installed successfully in Perkin-Elmer and Varian gas chromatographs.

The valve functions as follows: effluent from the chromatographic column is directed to entrance port 2 while purge gas of an equal flow rate enters port 1. Normally the flow from exit port 2 continues on to the detector and the flow from exit port 1 vents to the atmosphere. When shunting the flow from the detector the flow from entrance port 1 enters the detector and the entrance port 2 flow is vented, by way of exit port 3, to the atmosphere. The two operating positions are outlined in Figure 1.

Dual columns can be connected to a single detector in the usual manner with or without installation of a by-pass valve. Installation of a by-pass valve in the Micro-Tek MT-220 has been applied to numerous detection systems including the following: Coulson Conductivity, Dohrmann Microcoulometer, Melpar flame photometric, flame ionization, thermionic, and electron capture.

Usual operation with electron capture results in 2% or less base line deflection with equilibration in less than five seconds. No change in standing current of electron capture detectors was observed during operation up to 220°C and 15 psi indicating leak free operation. Due to the extremely small dead volume no peak broadening is observed. Similar results are obtained for the Melpar flame photometric and Varian thermionic detectors. ■

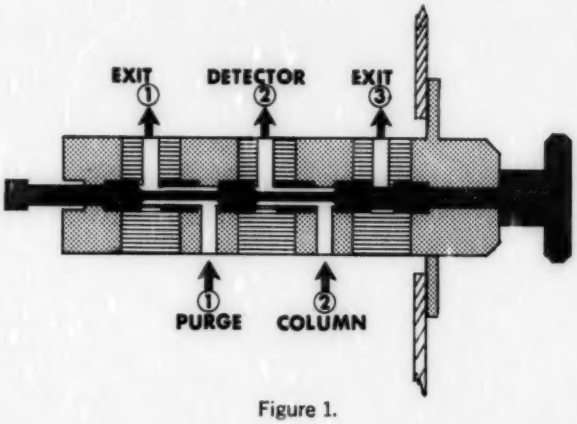

Figure 1.

Manuscript received July 22, 1967

Commercial sources and trade names are used for identification purposes only. Their mention does not constitute endorsement by the Public Health Services or by the U.S. Department of Health, Education, and Welfare.

**Five Port Linear Valve, Manual Model, No. GCL-M4005, Microtek Instruments, Inc., Baton Rouge, Louisiana.*

***Micro Volume Switching Valve, Manual Model, Cat. No. 2011, Carle Instruments, Inc., Anaheim, California.*

PB 275 114

JBLICATION

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STICIDES PROGRAM
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Determination of DDT and Metabolites, including DDA, in Human Urine by Gas Chromatography

by M. T. Cranmer, J. J. Carroll, and M. F. Copeland
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INTRODUCTION

The need to investigate the residues and metabolism of chlorinated hydrocarbons has stimulated the development of many analytical methods.[1-6] The earlier procedures utilizing colorimetry and bioassay[2] have, for the most part, given way to analysis by selective detector gas chromatography.[3-6] Currently, different biological tissues, fluids and excrement require separate clean-up and determinative analysis. Furthermore, these methods often necessitate separate analysis of polar and non-polar components.

This paper reports the development of a technique suitable for the routine analysis of DDT, DDE, DDD and free DDA present in human urine. The procedure includes joint electron-capture gas chromatographic determination of DDT, DDE, DDD, and DDA-methyl ester, as well as other extractable chlorinated hydrocarbons such as lindane, dieldrin and dichloran. Detection of these compounds is possible at levels as low as 1.0 ppb by the combination of the Coulson conductivity and parallel plate electron-capture detectors. Identification is facilitated by comparison of the relative responses of different detection systems and relative retention times on columns with different retention characteristics.

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Vol. 4, No. 4, 1969, published by Springer-Verlag New York Inc.

MATERIALS AND METHODS

Reagents: Hexane, petroleum ether, acetronitrile, toluene and acetone were either the Mallinkrodt* "Nanno-grade" or Burdick & Jackson "Distilled in Glass" products. Boron trifloride was obtained from the Matheson Co. in lecture bottles, and in nanograde Methanol reagent from Applied Science Laboratories. The coatings QF-1, DC-200, and SE-30 and the solid support Gas Chrom-Q were obtained from Applied Science Laboratories. Standard pesticides and pesticide metabolites were Food & Drug Administration Reference Standards.

Instruments: The gas chromatographs used were the Micro-Tek Model MT-220 fitted with an electron-capture detection system. One instrument was also equipped with the Coulson Conductivity detector, obtained from the Coulson Instrument Co. Measurement of osmolalities were made on an Osmette from Precision Systems.

Preparation of Glassware: When using electron-capture detection, thorough cleaning of glassware and the avoidance of plastics is mandatory in all steps of the procedure in order to avoid spurious peaks arising from sample contamination.

*Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Public Health Service.

Sample Collection and Preparation: Twenty-three individuals were selected to supply urine samples to test the method. They were divided into two approximately equal groups, the "general population" and "occupationally exposed".

Urine collections were made in sterile, screw-cap bottles to which 1 cc of toluene had been added as a preservative. Donors were requested to collect their specimens immediately after arising in the morning. The volume of urine routinely extracted varied for those individuals classified as normal (20-50 cc) and those with known or suspected exposure to unusual quantities of DDT or other chlorinated pesticides (5-10 cc).

The osmolality of each specimen was measured shortly after receipt using a Precision Systems Osmette.

Extraction Procedure: Each sample was mixed with equal volumes of 2% acetic acid in hexane by shaking the mixture in Teflon Stoppered separatory funnels for two minutes or by the use of an omni vortex mixer for samples in glass stoppered test tubes. When test tubes are used, centrifugation after mixing is desirable since any emulsion is eliminated. Test tubes were found to be more convenient for the extraction of 2- to 20-cc samples. Three equal volumes of 2% acetic acid in hexane extractant were used with each being equal to the volume of the urine sample.

In cases where persistant emulsions appeared, a few drops of acetonitrile were added to obviate the problem. The extracts were combined and evaporated to dryness in vacuo at 40-50°C, taking care that no residual traces of water remained (See Table I for recovery information).

Clean-up: Occasionally samples of urine with "general population" levels produced extracts containing sufficient amount of materials other than chlorinated hydrocarbons to warrant a "clean-up" step if electron capture determination was to be made. For this purpose the extract, after methylation and volume adjustment, was passed over a micro florisil column, according to the procedure of Enos and his co-workers.(9) This results in the removal of many interfering substances and the partition of the chlorinated pesticides present.

Esterification of Extracts: Each dry extract was treated with 2-3 cc of fresh methylation reagent, 10% boron triflouride in Nanograde Methanol. The reagent-sample mixture was heated at 50°C for 30 minutes in open tubes. To quench the reaction, 5 cc of distilled water was added to each tube. The resulting mixture was extracted three times with 5 cc volumes of hexane. The combined hexane extracts, after being reduced in vacuo and adjusted to the appropriate volume (usually 2.0cc), were then ready for analysis by electron-capture gas chromatography.

TABLE I

RECOVERIES FROM URINE SPIKED AT THREE LEVELS UPON EXTRACTION WITH 2% ACETIC ACID IN HEXANE FOLLOWED BY METHYLATION

Pesticide	I	II	III
Aldrin	91-95 93	91-95 94	92-96 95
Dieldrin	92-96 94	93-97 94	94-98 96
p,p'-DDA	91-95 93	90-94 93	93-96 95
p,p'-DDE	93-97 94	92-96 94	92-99 96
p,p'-DDD	92-96 94	94-97 95	95-100 97
p,p'-DDT	93-97 95	93-100 96	94-99 96
o,p'-DDT	93-97 94	94-98 95	93-101 97
o,p'-DDE	89-95 91	88 95 91	90-97 93
Endrin	92-96 93	92-97 93	95-102 97
Lindane	92-96 94	92-96 94	95-100 97

I. 10ppb, II. 25ppb, III. 100ppb

Results are reported as the Range and Average obtained upon analysis of four identical samples.

TABLE II

LEVELS OF DDT-DERIVED MATERIALS IN HUMAN URINE IN P. P. B.

Pesticide	General Population		Occupationally Exposed	
	Range	Mean	Range	Mean
p,p'-DDA	8.1-19.0	14.2	33.0-148	75.3
p,p'-DDT	0.48-0.67	0.36	0.80-7.2	2.90
p,p'-DDE	0.41-0.55	0.42	1.10-1.3	1.20
p,p'-DDD	0.25-0.43	0.34	0.89-1.4	1.10

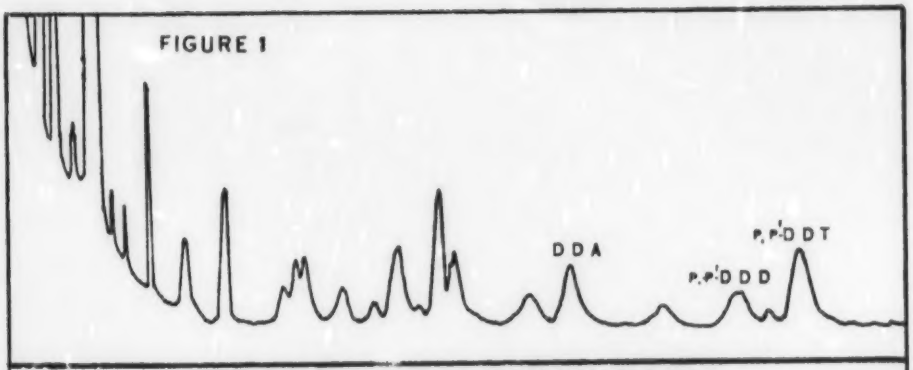
Analysis by Gas-Liquid Chromatography: Micro-Tek Model MT-220 gas chromatographs, equipped with electron capture or Coulson Conductivity systems, were used throughout the study. The Dohrmann microcoulometer detection system can be used in place of the Coulson conductivity system. These systems are roughly equal in their sensitivity to chlorinated hydrocarbons.

The most useful column type proved to be a 6'x1/4" O.D. Pyrex glass U-tube packed with 5% QF-1 on 80/100 mesh Gas Chrom-Q. Conditions for chromatography using 5% QF-1 columns were an oven temperature of 170-175°C and a nitrogen carrier flow of 80-100 cc/min. Other successful column packings were 5% QF-1 + 7.5% DC-200 and 3% SE-30 on 80/100 mesh Gas Chrom-Q.

RESULTS

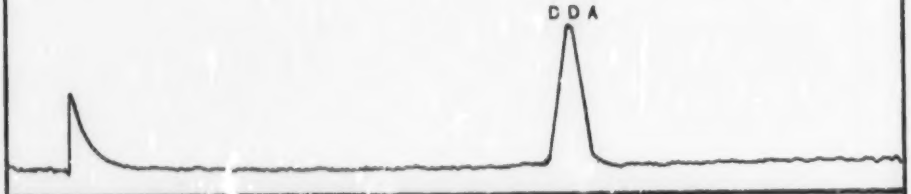
The differences in range of the two exposure groups are not as great as one might expect, however table II shows that mean values, particularly for DDA, are different. All values obtained from urinalysis were corrected to an osmolality of 1000 milliosmols by multiplying the observed value times a correction factor, K = 1000/observed osmolality. Satisfactory results are usually obtained, especially for the occupationally exposed, via the selective extraction method described and the use of an extra "clean-up" step is not always necessary. However, especially when examining lower level general population samples, materials which

FIGURE 1



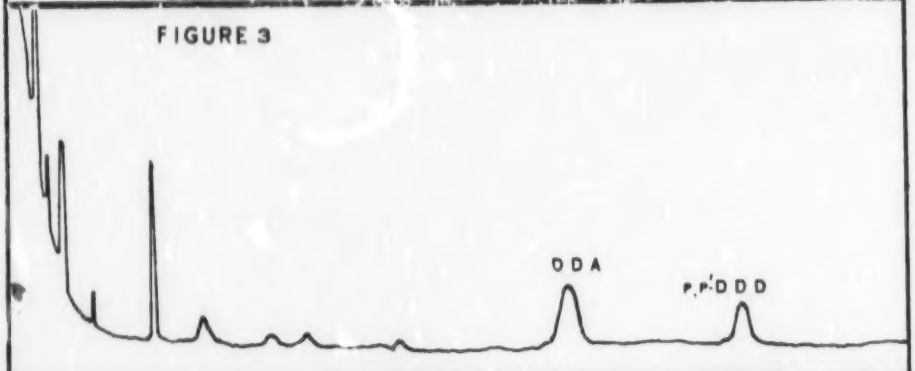
EXAMPLE OF A POOR EC CHROMATOGRAM OF A GENERAL POPULATION URINE EXTRACT

FIGURE 2



EXAMPLE OF COULSON CONDUCTIVITY CHROMATOGRAM OBTAINED BY CONCENTRATING EXTRACT SHOWN IN FIGURE 1

FIGURE 3



EXAMPLE OF AN EC CHROMATOGRAM OF FRACTION 2 FROM THE MICRO FLORISIL CLEAN UP OF THE EXTRACT SHOWN IN FIGURE 1

may not be chlorinated pesticides are extracted from the urine and interfere with analysis by resulting in electron-capture chromatograms too complicated for easy peak assignment (Figure 1). In these cases the simplest, most straight-forward method of analysis was found to be concentration of the extract to an appropriate volume (often about 0.20cc) with subsequent conductivity or microcoulometric detection (Figure 2). However, if electron-capture detection is to be used, clean-up by the micro florisil column is necessary (Figure 3).

DISCUSSION

The procedure described combines several desirable features including simplicity, speed of analysis, reproducibility and produces chromatograms with a minimum of interference. The lower limit level of detectability of the various chlorinated pesticides and metabolites can be adjusted by increasing the volume of urine originally extracted, concentrating the extracts to smaller volumes, injecting larger aliquots into gas chromatographs equipped with conductivity or microcoulometric detection systems, or by the inclusion of the micro-florisil clean-up step. One of the problems encountered in this procedure is the inherent lack of sensitivity of the methyl ester of DDA to electron-capture detection. In no case was it possible to detect the ester in a concentration of less than 2 ng.

total injected, whereas p,p'-DDT could be detected at the level of 50 pg. or less. In certain cases use of the conductivity or microcoulometric detectors was particularly valuable since sensitivity to chlorinated pesticides varies only with chlorine content. These detectors therefore will "see" the methyl ester of DDA while ignoring DDT and its other metabolites which are usually present in much lower concentrations.

It must be emphasized that the levels of pesticides reported for the two groups referred to as "general population" and "occupationally exposed" should not necessarily be considered representive of like groups throughout the country. The results obtained here are reported to demonstrate a method and not as a study of populational levels. However, it can be seen easily that the occupationally exposed group has urine DDA levels approximately ten times that of the general population group.

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PB 275 115

Relative Retention Ratios of Ninety-Five Pesticides and Metabolites on Nine Gas-Liquid Chromatographic Columns Over a Temperature Range of 170 to 204°C in Two Detection Modes

PESTICIDE PUBLICATION

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710

Environmental Protection Agency

Tables of relative retention data are presented for 95 organochlorine and organophosphorus pesticidal compounds at column oven temperatures from 170 to 204°C in 2° increments. Retention values, relative to aldrin or parathion ($RRT_{A\ or\ P}$), were found to vary inversely with increasing column temperature for all compounds eluting later than the reference compound, and in direct proportion for those compounds eluting earlier than the fixed reference compound. Studies revealed a linear relationship between column temperature and relative retention ratios; thus, by obtaining precise values by gas-liquid chromatography for a number of pesticidal compounds at 170, 185, and 200°C, a computer program was developed to obtain $RRT_{A\ or\ P}$ values at all 2° increments from 170 to 204°C, the customary limits in the gas-liquid chromatography of pesticides. The tables are valuable in the tentative identification of pesticidal peaks resulting from the chromatography of sample extracts for multiresidue assessment.

A number of authors have published relative retention data of pesticidal compounds obtained on various gas-liquid chromatographic (GLC) columns by different modes of detection (1–7). In each of the cited publications, the relative retention time (RRT) data have been obtained at one given temperature for each column used.

These data have proved valuable, provided that the researcher carefully adjusts the column temperature to the same point as that reported by the author. In some instances, this is difficult, since the readout pyrometer on many gas chromatographs may be grossly in error. When this occurs unbeknown to the chromatographer, the resulting RRT values, particularly those for late eluting compounds, will be significantly different from those reported in a publication. Therefore, if the chromatographer seeks to duplicate the published values, the column oven must be operated at the same temperature.

Trials chromatographing pesticidal compounds and metabolites in this laboratory, using electron capture and flame photometric detection (phosphorus mode), have shown conclusively that a linear relationship exists between column temperature and RRT over a temperature range of 170 to 204°C. The RRT of a given compound eluting later than the reference compound varies inversely with the increase in temperature, whereas the values of compounds eluting earlier than the reference compound generally vary in direct proportion to the temperature increase.

In view of these findings, the development of tables of RRT values applicable at any given temperature in the range of 170 to 204°C provides the pesticide chemist with precise and useful data, irrespective of the oven pyrometer readout at any time.

METHOD

Apparatus and Reagents

(a) *Gas chromatographic equipment.*—Tracor Model 220 gas chromatograph equipped with parallel plate, tritium source electron capture detector; Melpar flame photometric detector with 526 nm filter; oven accommodating up to 4 U-shaped columns, each 1.829 m (6') × 4 mm id; Vycor glass injection port "demister" tube for off-column injections.

(b) *Computer.*—Hewlett-Packard Model 2100A equipped with teleprinter readout.

(c) *Thermometers.*—Mercury, precision, −1 to 201°C, in 0.2° graduations, 610 mm long, National Bureau of Standards (NBS) calibrated; mercury, 0–250°C, graduated in 1° subdivisions, accuracy ±0.5°C.

(d) *Standards.*—Standard pesticide mixtures of

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up to 13 compounds, none of which would produce peak overlaps with any other component compound. Appropriate dilutions made with isooctane.

(e) *Column conditioner.*—Silyl 8, 1.0 ml ampoules (Pierce Chemical Co., Rockford, IL 61105)

Column Packings

All column packing materials were purchased precoated, on quality specifications, from commercial suppliers. The materials coated on Chromosorb W supports were purchased from Supelco, Inc., Bellefonte, PA 16823, and those coated on Gas-Chrom Q from Applied Science Laboratories, Inc., State College, PA 16801.

The GLC columns selected for the development of these data were as follows:

(*1*) Via electron capture detection:

5% OV-210 on 100–120 mesh Chromosorb W (HP)

10% DC-200 on 80–100 mesh Chromosorb W (HP)

4% SE-30/6% QF-1 on 80–100 mesh Chromosorb W (HP)

1.5% OV-17/1.95% QF-1 on 100–120 mesh Chromosorb W (HP)

5% DC-200/7.5% QF-1 on 80–100 mesh Chromosorb W (HP)

1.6% OV-17/6.4% OV-210 on 80–100 mesh Gas-Chrom Q

(*2*) Via flame photometric detection:

10% OV-210 on 100–120 mesh Gas-Chrom Q

4% SE-30/6% OV-210 on 80–100 mesh Gas-Chrom Q

1.5% OV-17/1.95% OV-210 on 100–120 mesh Gas-Chrom Q

Column Preparation and Evaluation

Each column, after it was packed and the ends were plugged with silanized glass wool, was heat-conditioned by placing it in the GLC oven with the inlet end connected to the injection port and with the outlet end vented inside the oven. Conditioning was carried out at 245°C for 72–96 hr with a 60 ml carrier gas (nitrogen)/ml flow through the column.

Following the heat-conditioning period, the oven temperature was reduced to the operating level. Each column to be used for chlorinated compounds (electron capture detection) received 3 consecutive injections of 25 μl each of Silyl 8. The injections were spaced ca 30 min apart. The effluent was vented into the oven for $\geq$3 hr after the final injection and before the outlet end of the column was connected to the detector transfer line.

Each of the columns of mixed liquid phases was carefully evaluated to be certain that the stated proportions of liquid phase materials were correct. This was carried out by adjusting the oven temperature to precisely 200±0.5°C and chromatographing a mixture of 13 chlorinated pesticides. Using the retention of aldrin as the reference point, the RRT_A was computed for all other compounds. From past research in this laboratory (4), the correct RRT_A values for all compounds at the given temperature were known so that values obtained on the new columns could be compared.

Adjustment and Calibration of Column Temperature

To ensure maximum validity, it was necessary to monitor column oven temperature more accurately than is often possible with the built-in pyrometer. A long-stem, NBS-calibrated glass thermometer was inserted into one of the unused injection ports and was lowered until the mercury bulb was at the center location of the columns. The oven temperature was adjusted to precisely 200±0.2°C. This was held ca 3 hr to ensure equilibration. The thermometer was then raised until the mercury bulb reached the same vertical point, ca 5″ from the top of the oven, where the bulb of a lower quality glass thermometer would be placed throughout the study. The temperature differential never exceeded 0.4°C, with a higher reading being recorded near the top of the oven, presumably due to heat radiated from the 225°C injection block. The lower quality thermometer, previously calibrated against the NBS one, was then inserted and the temperature reading was logged. Any subsequent oven temperature adjustment required during this portion of the study was targeted to the initial reading of the thermometer used as the continuous monitor.

Upon completion of the foregoing calibration, the instrument pyrometer was adjusted to coincide with the established temperature so that both pyrometer and mercury thermometer were reading identically.

Discussion

In the development of the data for Tables 1–9,[2] 18 RRT values are given for each compound at 2°C intervals. Obviously, the task of determining RRT values at each of the 18 temperature intervals would be overwhelming. It was recognized from previously obtained data, using both electron capture and flame photometric detectors, that a linear relationship existed between column temperature and RRT. Figure 1

[2] In Tables 1–9, ME = methyl esters, IPE = isopropyl esters, and BE = butyl esters.

Table 1. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 5% OV-210 on 100–120 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 50 ml/min

170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	
0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	Mevinphos
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.68	0.69	2,4-D (ME)
0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.65	0.65	0.65	0.65	0.65	0.65	0.66	0.66	0.66	0.66	0.66	Phorate
0.62	0.62	0.62	0.63	0.63	0.64	0.64	0.65	0.65	0.65	0.66	0.66	0.67	0.67	0.68	0.68	0.68	0.69	α-BHC
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	CDEC
0.88	0.87	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.83	0.83	0.83	0.82	2,4-D (IPE)
0.87	0.86	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.82	0.81	Simazine
0.88	0.88	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.84	0.84	0.84	0.83	0.83	0.82	0.82	0.81	0.81	Atrazine
0.76	0.75	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	Diazinon
0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.84	Lindane
1.08	1.08	1.07	1.07	1.06	1.06	1.05	1.04	1.03	1.03	1.02	1.01	1.01	1.00	1.00	0.99	0.98	0.98	2,4,5-T (ME)
0.97	0.97	0.96	0.96	0.96	0.96	0.96	0.95	0.95	0.95	0.95	0.95	0.95	0.94	0.94	0.94	0.94	0.94	β-BHC
1.30	1.29	1.28	1.27	1.26	1.25	1.23	1.22	1.21	1.20	1.19	1.18	1.16	1.15	1.14	1.13	1.12	1.11	2,4-D (BE) I
1.07	1.07	1.06	1.06	1.06	1.05	1.05	1.04	1.04	1.03	1.03	1.03	1.02	1.02	1.01	1.01	1.01	1.00	δ-BHC
0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	Heptachlor
1.36	1.34	1.33	1.32	1.30	1.29	1.27	1.26	1.25	1.23	1.22	1.20	1.19	1.18	1.16	1.15	1.14	1.12	2,4,5-T (IPE)
1.47	1.46	1.44	1.43	1.41	1.40	1.38	1.36	1.35	1.33	1.32	1.30	1.29	1.27	1.25	1.24	1.22	1.21	2,4-D (BE) II
1.51	1.51	1.50	1.49	1.48	1.48	1.47	1.46	1.45	1.45	1.44	1.43	1.42	1.42	1.41	1.40	1.39	1.39	Dichlone
2.18	2.15	2.12	2.09	2.06	2.04	2.01	1.98	1.95	1.92	1.89	1.86	1.83	1.80	1.78	1.75	1.72	1.69	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.41	1.40	1.39	1.38	1.37	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.24	Ronnel
1.43	1.42	1.41	1.40	1.39	1.38	1.38	1.37	1.36	1.35	1.34	1.33	1.33	1.32	1.31	1.30	1.29	1.28	1-Hydroxychlordene
3.17	3.12	3.07	3.02	2.97	2.92	2.88	2.83	2.78	2.73	2.68	2.63	2.58	2.54	2.49	2.44	2.39	2.34	Methyl parathion
2.02	2.00	1.98	1.97	1.95	1.93	1.91	1.89	1.87	1.85	1.83	1.81	1.79	1.77	1.75	1.73	1.71	1.69	Heptachlor epoxide
3.20	3.14	3.08	3.02	2.96	2.91	2.85	2.79	2.73	2.67	2.61	2.55	2.49	2.44	2.38	2.32	2.26	2.20	Malathion
2.84	2.80	2.75	2.71	2.67	2.63	2.59	2.54	2.50	2.46	2.41	2.37	2.33	2.29	2.24	2.20	2.16	2.12	DCPA
2.06	2.04	2.02	1.99	1.97	1.94	1.91	1.89	1.87	1.84	1.81	1.79	1.77	1.74	1.71	1.69	1.66	1.64	Dyrene
1.67	1.65	1.63	1.62	1.60	1.59	1.57	1.55	1.53	1.52	1.50	1.49	1.47	1.45	1.43	1.42	1.40	1.39	o,p'-DDE
1.97	1.95	1.93	1.90	1.88	1.86	1.84	1.82	1.80	1.78	1.75	1.73	1.71	1.69	1.67	1.65	1.63	1.61	Chlorbenside
4.24	4.17	4.09	4.02	3.94	3.87	3.78	3.71	3.63	3.56	3.48	3.40	3.33	3.25	3.17	3.10	3.03	2.95	Ethyl parathion
2.63	2.60	2.57	2.54	2.51	2.48	2.45	2.42	2.39	2.36	2.33	2.30	2.27	2.24	2.21	2.18	2.15	2.12	Endosulfan I
2.24	2.21	2.18	2.15	2.12	2.10	2.07	2.04	2.01	1.98	1.96	1.92	1.90	1.87	1.84	1.81	1.78	1.75	p,p'-DDE
3.10	3.05	3.00	2.94	2.89	2.84	2.79	2.74	2.69	2.64	2.59	2.54	2.48	2.43	2.38	2.33	2.28	2.23	DDA (ME)
4.47	4.40	4.32	4.24	4.17	4.09	4.01	3.93	3.85	3.77	3.69	3.62	3.54	3.46	3.38	3.30	3.22	3.14	Captan
4.04	3.98	3.92	3.85	3.78	3.72	3.65	3.59	3.53	3.46	3.39	3.33	3.26	3.19	3.13	3.06	2.99	2.93	Folpet
3.21	3.17	3.13	3.09	3.04	3.00	2.96	2.92	2.88	2.83	2.79	2.75	2.71	2.68	2.62	2.58	2.54	2.49	Dieldrin
2.07	2.04	2.01	1.98	1.96	1.92	1.89	1.86	1.83	1.80	1.77	1.74	1.71	1.68	1.65	1.62	1.59	1.56	Perthane
2.74	2.70	2.67	2.63	2.59	2.55	2.51	2.47	2.43	2.39	2.35	2.31	2.27	2.23	2.19	2.15	2.11	2.07	o,p'-DDD
2.92	2.87	2.83	2.79	2.74	2.70	2.66	2.61	2.57	2.52	2.48	2.43	2.39	2.35	2.30	2.26	2.21	2.17	o,p'-DDT
3.82	3.77	3.72	3.67	3.61	3.56	3.50	3.45	3.39	3.34	3.28	3.23	3.18	3.13	3.07	3.02	2.96	2.91	Endrin
2.76	2.73	2.70	2.67	2.64	2.61	2.58	2.56	2.52	2.49	2.46	2.43	2.40	2.37	2.34	2.31	2.28	2.25	Chlordecone
4.10	4.03	3.96	3.89	3.82	3.75	3.68	3.61	3.53	3.46	3.38	3.32	3.25	3.18	3.11	3.03	2.96	2.89	p,p'-DDD
4.98	4.91	4.83	4.75	4.67	4.59	4.51	4.43	4.35	4.27	4.19	4.11	4.03	3.95	3.87	3.79	3.71	3.63	Endosulfan II
6.0	5.89	5.78	5.64	5.51	5.37	5.25	5.12	4.99	4.86	4.73	4.60	4.48	4.35	4.22	4.09	3.96	3.83	Ethion
4.47	4.39	4.31	4.23	4.15	4.07	3.98	3.90	3.82	3.74	3.66	3.58	3.49	3.41	3.33	3.25	3.17	3.09	p,p'-DDT
5.29	5.19	5.09	4.99	4.89	4.78	4.68	4.58	4.48	4.37	4.27	4.17	4.06	3.96	3.86	3.76	3.16	3.06	Carbophenothion
12.3	12.0	11.7	11.4	11.1	10.8	10.4	10.1	9.8	9.5	9.2	8.9	8.6	8.2	7.9	7.6	7.3	7.0	Dilan I
4.06	4.01	3.96	3.89	3.84	3.78	3.73	3.68	3.62	3.57	3.52	3.46	3.40	3.35	3.29	3.24	3.19	3.13	Mirex
7.4	7.2	7.0	6.8	6.7	6.5	6.3	6.1	5.94	5.76	5.59	5.41	5.23	5.06	4.88	4.70	4.53	4.35	Methoxychlor
14.2	13.8	13.4	13.0	12.6	12.3	11.9	11.5	11.1	10.7	10.3	10.0	9.6	9.2	8.8	8.4	8.1	7.7	Dilan II
21.1	20.6	20.0	19.5	18.9	18.4	17.9	17.3	16.7	16.2	15.6	15.1	14.5	14.0	13.4	12.9	12.3	11.8	Tetradifon
24.7	24.0	23.3	22.6	21.9	21.2	20.6	19.9	19.2	18.5	17.8	17.1	16.4	15.7	15.0	14.3	13.6	12.9	Azinphosmethyl
170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	

Table 2. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 10% DC-200 on 80–100 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 120 ml/min

170	174	178	182	186	190	194	198	202	204	
0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	Mevinphos
0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	2,4-D (ME)
0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.38	Phorate
0.35	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.39	0.39	α-BHC
0.35	0.35	0.36	0.36	0.37	0.37	0.37	0.38	0.38	0.39	CDEC
0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	0.41	2,4-D (IPE)
0.39	0.39	0.39	0.39	0.40	0.40	0.40	0.40	0.41	0.41	Simazine
0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.43	0.43	Atrazine
0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	Diazinon
0.43	0.43	0.44	0.44	0.45	0.45	0.46	0.46	0.47	0.47	Lindane
0.48	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	2,4,5-T (ME)
0.38	0.38	0.39	0.39	0.40	0.40	0.41	0.41	0.42	0.42	β-BHC
0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	2,4-D (BE) I
0.45	0.45	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.49	δ-BHC
0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79	0.80	0.80	Heptachlor
0.67	0.67	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66	2,4,5-T (IPE)
0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	2,4-D (BE) II
0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	Dichlone
0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.46	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
0.82	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	Ronnel
1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	1.00	1.00	1-Hydroxychlordene
0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	0.69	Methyl parathion
1.29	1.29	1.28	1.28	1.27	1.27	1.26	1.26	1.25	1.24	Heptachlor epoxide
0.77	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.79	0.80	Malathion
1.10	1.09	1.09	1.08	1.08	1.07	1.06	1.06	1.05	1.05	DCPA
1.29	1.28	1.27	1.27	1.26	1.25	1.24	1.24	1.23	1.22	Dyrene
1.65	1.64	1.62	1.61	1.59	1.58	1.57	1.55	1.54	1.53	o,p'-DDE
1.43	1.42	1.41	1.40	1.40	1.39	1.38	1.37	1.36	1.36	Chlorbenside
1.03	1.02	1.02	1.01	1.00	1.00	0.99	0.99	0.98	0.98	Ethyl parathion
1.64	1.63	1.62	1.61	1.61	1.60	1.59	1.58	1.58	1.57	Endosulfan I
2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.94	1.92	1.90	p,p'-DDE
1.81	1.79	1.77	1.75	1.73	1.71	1.69	1.67	1.65	1.63	DDA (ME)
1.21	1.21	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	Captan
1.30	1.29	1.29	1.28	1.27	1.27	1.26	1.26	1.25	1.24	Folpet
1.99	1.98	1.96	1.95	1.93	1.92	1.90	1.89	1.87	1.86	Dieldrin
2.60	2.56	2.53	2.49	2.45	2.41	2.38	2.34	2.30	2.27	Perthane
2.13	2.11	2.08	2.06	2.04	2.01	1.99	1.97	1.94	1.92	o,p'-DDD
2.90	2.86	2.83	2.79	2.76	2.72	2.68	2.65	2.61	2.58	o,p'-DDT
2.22	2.20	2.19	2.17	2.16	2.14	2.13	2.11	2.09	2.08	Endrin
3.07	3.03	3.00	2.96	2.93	2.90	2.86	2.83	2.79	2.76	Chlordecone
2.72	2.69	2.66	2.62	2.59	2.56	2.52	2.49	2.45	2.42	p,p'-DDD
2.29	2.27	2.25	2.24	2.22	2.20	2.18	2.17	2.15	2.13	Endosulfan II
3.04	3.00	2.95	2.91	2.86	2.81	2.77	2.72	2.68	2.63	Ethion
3.72	3.67	3.62	3.57	3.51	3.46	3.40	3.35	3.29	3.24	p,p'-DDT
3.42	3.37	3.32	3.26	3.21	3.16	3.11	3.06	3.01	2.96	Carbophenothion
3.23	3.19	3.15	3.11	3.06	3.02	2.98	2.93	2.89	2.85	Dilan I
6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	Mirex
6.1	6.0	5.89	5.77	5.66	5.54	5.42	5.30	5.18	5.06	Methoxychlor
4.07	4.02	3.98	3.89	3.83	3.77	3.70	3.64	3.58	3.52	Dilan II
6.4	6.3	6.2	6.1	5.97	5.85	5.73	5.62	5.50	5.38	Tetradifon
6.4	6.3	6.2	6.0	5.92	5.81	5.69	5.58	5.46	5.34	Azinphosmethyl
170	174	178	182	186	190	194	198	202	204	
0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.19	0.20	Mevinphos
0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	2,4-D (ME)
0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.40	0.41	Phorate
0.39	0.39	0.39	0.40	0.40	0.41	0.41	0.42	0.42	0.43	α-BHC
0.39	0.39	0.39	0.40	0.40	0.41	0.41	0.41	0.41	0.42	CDEC
0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.43	0.44	2,4-D (IPE)
0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.42	0.42	0.42	Simazine
0.43	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44	0.44	Atrazine
0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	Diazinon
0.47	0.47	0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	Lindane
0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.50	2,4,5-T (ME)
0.42	0.42	0.43	0.43	0.44	0.44	0.45	0.45	0.46	0.46	β-BHC
0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	2,4-D (BE) I
0.49	0.49	0.49	0.50	0.50	0.51	0.51	0.51	0.51	0.52	δ-BHC
0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.82	Heptachlor
0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	2,4,5-T (IPE)
0.70	0.70	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	2,4-D (BE) II
0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.53	0.53	0.53	Dichlone
0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
0.80	0.79	0.79	0.79	0.78	0.78	0.78	0.78	0.78	0.78	Ronnel
1.00	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.98	1-Hydroxychlordene
0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	Methyl parathion
1.24	1.23	1.23	1.22	1.22	1.21	1.20	1.20	1.19	1.18	Heptachlor epoxide
0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	Malathion
1.04	1.03	1.03	1.02	1.02	1.01	1.01	1.00	1.00	0.99	DCPA
1.22	1.21	1.20	1.20	1.19	1.18	1.17	1.17	1.16	1.15	Dyrene
1.51	1.50	1.48	1.47	1.46	1.44	1.43	1.41	1.40	1.39	o,p'-DDE
1.35	1.34	1.33	1.33	1.32	1.31	1.30	1.30	1.29	1.28	Chlorbenside
0.97	0.96	0.96	0.95	0.95	0.94	0.93	0.93	0.92	0.92	Ethyl parathion
1.56	1.55	1.54	1.54	1.53	1.52	1.51	1.50	1.49	1.48	Endosulfan I
1.88	1.86	1.83	1.81	1.79	1.77	1.75	1.73	1.71	1.69	p,p'-DDE
1.61	1.60	1.58	1.56	1.54	1.52	1.50	1.48	1.46	1.44	DDA (ME)
1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.14	1.13	1.12	Captan
1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.20	1.20	1.19	Folpet
1.84	1.83	1.81	1.80	1.79	1.77	1.76	1.74	1.73	1.72	Dieldrin
2.23	2.19	2.15	2.11	2.08	2.04	2.00	1.97	1.94	1.91	Perthane
1.90	1.87	1.85	1.83	1.80	1.78	1.76	1.73	1.71	1.69	o,p'-DDD
2.54	2.50	2.47	2.43	2.40	2.38	2.32	2.29	2.26	2.23	o,p'-DDT
2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	2.46	2.43	Endrin
2.76	2.72	2.69	2.66	2.62	2.59	2.55	2.52	2.49	2.46	Chlordecone
2.42	2.39	2.35	2.32	2.29	2.26	2.22	2.19	2.15	2.12	p,p'-DDD
2.13	2.11	2.10	2.08	2.06	2.05	2.03	2.01	2.00	1.98	Endosulfan II
2.63	2.58	2.54	2.49	2.44	2.40	2.35	2.31	2.26	2.22	Ethion
3.24	3.18	3.13	3.08	3.02	2.97	2.92	2.86	2.81	2.76	p,p'-DDT
2.96	2.90	2.85	2.80	2.75	2.70	2.65	2.60	2.55	2.50	Carbophenothion
2.89	2.85	2.80	2.76	2.72	2.67	2.63	2.58	2.54	2.50	Dilan I
6.2	6.1	6.0	5.90	5.80	5.69	5.59	5.48	5.38	5.28	Mirex
5.30	5.18	5.06	4.93	4.82	4.69	4.58	4.45	4.33	4.22	Methoxychlor
3.64	3.58	3.52	3.46	3.39	3.33	3.27	3.20	3.14	3.08	Dilan II
5.62	5.50	5.38	5.27	5.15	5.03	4.92	4.80	4.68	4.57	Tetradifon
5.58	5.46	5.34	5.23	5.12	5.00	4.88	4.77	4.66	4.54	Azinphosmethyl
170	174	178	182	186	190	194	198	202	204	

Table 3. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 1.5% OV-17/ 1.95% QF-1 on 100–120 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 60 ml/min

170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	
0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	Mevinphos
0.44	0.45	0.45	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	2,4-D (ME)
0.48	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.52	Phorate
0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.54	0.54	0.54	0.55	*α*-BHC
0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	CDEC
0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	2,4-D (IPE)
0.70	0.70	0.70	0.70	0.69	0.69	0.69	0.69	0.69	0.69	0.68	0.68	0.68	0.68	0.67	0.67	0.67	0.67	0.67	Simazine
0.69	0.69	0.69	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.66	Atrazine
0.67	0.67	0.68	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	Diazinon
0.66	0.67	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	Lindane
0.76	0.76	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	2,4,5-T (ME)
0.82	0.82	0.82	0.82	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.80	*β*-BHC
0.86	0.86	0.85	0.84	0.84	0.84	0.83	0.83	0.82	0.82	0.81	0.81	0.80	0.80	0.79	0.79	0.78	0.78	2,4-D (BE) I
0.97	0.97	0.97	0.96	0.96	0.96	0.95	0.95	0.94	0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.92	0.91	*δ*-BHC
0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	Heptachlor
0.94	0.94	0.93	0.92	0.92	0.91	0.90	0.90	0.89	0.88	0.88	0.87	0.87	0.86	0.85	0.85	0.84	0.83	2,4,5-T (IPE)
1.03	1.02	1.01	1.01	1.00	0.99	0.99	0.98	0.97	0.96	0.96	0.95	0.94	0.93	0.93	0.92	0.91	0.90	2,4-D (BE) II
1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.98	Dichlone
1.17	1.16	1.15	1.14	1.13	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.03	1.02	1.01	1.00	0.99	0.98	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.17	1.16	1.16	1.15	1.14	1.14	1.13	1.12	1.12	1.11	1.10	1.09	1.09	1.08	1.07	1.07	1.06	1.05	Ronnel
1.41	1.40	1.39	1.38	1.36	1.35	1.34	1.33	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	1.24	1.23	1-Hydroxychlordene
1.71	1.69	1.67	1.66	1.64	1.62	1.60	1.59	1.57	1.55	1.53	1.52	1.50	1.48	1.47	1.45	1.43	1.41	Methyl parathion
1.70	1.69	1.68	1.67	1.66	1.65	1.64	1.63	1.62	1.61	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	Heptachlor epoxide
2.07	2.04	2.01	1.98	1.95	1.92	1.89	1.87	1.84	1.81	1.78	1.75	1.72	1.69	1.66	1.63	1.60	1.57	Malathion
1.82	1.80	1.78	1.76	1.74	1.72	1.70	1.68	1.66	1.64	1.62	1.60	1.58	1.56	1.54	1.52	1.50	1.48	DCPA
2.15	2.13	2.11	2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.94	1.91	1.89	1.87	1.85	1.83	1.81	1.79	Dyrene
2.14	2.12	2.09	2.07	2.05	2.03	2.01	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	1.79	1.77	o,p'-DDE
2.20	2.18	2.16	2.14	2.12	2.10	2.08	2.06	2.05	2.03	2.01	1.99	1.97	1.95	1.93	1.91	1.89	1.87	Chlorbenside
2.32	2.25	2.25	2.22	2.19	2.16	2.13	2.09	2.08	2.03	2.00	1.97	1.93	1.90	1.87	1.84	1.81	1.78	Ethyl parathion
2.20	2.18	2.16	2.15	2.13	2.11	2.10	2.08	2.06	2.05	2.03	2.01	2.00	1.98	1.97	1.95	1.93	1.91	Endosulfan I
2.75	2.72	2.69	2.64	2.61	2.58	2.54	2.51	2.47	2.43	2.40	2.37	2.33	2.30	2.27	2.23	2.20	2.17	*p,p'*-DDE
2.97	2.93	2.89	2.84	2.79	2.75	2.71	2.66	2.62	2.57	2.53	2.49	2.44	2.40	2.35	2.31	2.27	2.22	DDA (ME)
3.27	3.22	3.18	3.13	3.07	3.04	3.00	2.95	2.91	2.86	2.81	2.77	2.73	2.68	2.63	2.59	2.54	2.50	Captan
3.32	3.27	3.23	3.18	3.13	3.08	3.04	2.99	2.95	2.91	2.87	2.82	2.77	2.73	2.68	2.64	2.59	2.55	Folpet
2.80	2.77	2.75	2.72	2.69	2.67	2.64	2.61	2.59	2.56	2.53	2.51	2.48	2.46	2.43	2.40	2.37	2.35	Dieldrin
3.52	3.46	3.41	3.35	3.30	3.24	3.19	3.14	3.08	3.03	2.98	2.92	2.87	2.82	2.76	2.71	2.66	2.60	Perthane
3.34	3.29	3.25	3.20	3.15	3.11	3.06	3.01	2.97	2.92	2.88	2.83	2.77	2.74	2.69	2.65	2.60	2.56	o,p'-DDD
3.98	3.94	3.88	3.83	3.77	3.71	3.66	3.60	3.54	3.48	3.43	3.38	3.32	3.27	3.21	3.16	3.10	3.04	o,p'-DDT
3.47	3.43	3.40	3.36	3.33	3.29	3.26	3.22	3.18	3.15	3.12	3.08	3.04	3.01	2.97	2.93	2.90	2.87	Endrin
3.26	3.23	3.19	3.16	3.13	3.09	3.06	3.03	3.00	2.96	2.93	2.90	2.87	2.83	2.80	2.77	2.74	2.70	Chlordecone
4.65	4.57	4.49	4.41	4.33	4.26	4.18	4.10	4.02	3.94	3.87	3.79	3.71	3.64	3.61	3.48	3.40	3.32	*p,p'*-DDD
4.45	4.39	4.34	4.28	4.23	4.17	4.11	4.05	3.99	3.94	3.88	3.82	3.76	3.71	3.65	3.59	3.54	3.48	Endosulfan II
6.1	5.97	5.85	5.73	5.61	5.49	5.36	5.24	5.12	5.00	4.88	4.76	4.64	4.52	4.40	4.28	4.16	4.04	Ethion
5.57	5.48	5.39	5.29	5.20	5.11	5.01	4.92	4.83	4.74	4.64	4.55	4.46	4.36	4.27	4.18	4.09	4.00	*p,p'*-DDT
6.4	6.2	6.1	5.99	5.88	5.76	5.64	5.52	5.40	5.28	5.16	5.04	4.92	4.80	4.68	4.56	4.44	4.32	Carbophenothion
8.0	7.9	7.7	7.5	7.4	7.2	7.1	6.9	6.7	6.6	6.4	6.3	6.1	5.95	5.80	5.62	5.48	5.34	Dilan I
7.7	7.6	7.5	7.3	7.3	7.1	7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	6.0	5.85	Mirex
12.4	12.1	11.8	11.6	11.3	11.0	10.7	10.4	10.1	9.8	9.5	9.3	9.0	8.7	8.4	8.1	7.8	7.5	Methoxychlor
9.4	9.2	9.0	8.8	8.6	8.4	8.2	8.0	7.8	7.6	7.3	7.1	6.9	6.7	6.5	6.3	6.1	5.88	Dilan II
16.9	16.5	16.1	15.7	15.3	14.9	14.5	14.1	13.7	13.3	12.9	12.5	12.1	11.7	11.3	10.9	10.5	10.2	Tetradifon
24.0	23.4	22.8	22.2	21.6	21.0	20.4	19.8	19.2	18.6	18.0	17.4	16.8	16.2	15.6	15.0	14.4	13.8	Azinphosmethyl
170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	

Table 4. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 4% SE-30/6% QF-1 on 80–100 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml/min

170		174		178		182		186		190		194		198		202	204	
0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.33	Mevinphos
0.39	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	0.44	0.44	0.45	2,4-D (ME)
0.43	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.48	Phorate
0.42	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.50	α-BHC
0.44	0.44	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.50	0.50	0.50	0.51	CDEC
0.54	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	2,4-D (IPE)
0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.54	0.54	0.54	Simazine
0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.57	0.57	0.57	Atrazine
0.60	0.60	0.60	0.60	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.58	0.58	0.58	0.58	0.58	0.58	0.58	Diazinon
0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	Lindane
0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	0.64	2,4,5-T (ME)
0.57	0.57	0.57	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	β-BHC
0.85	0.84	0.84	0.83	0.83	0.82	0.82	0.82	0.81	0.81	0.80	0.80	0.79	0.79	0.79	0.78	0.78	0.77	2,4-D (BE) I
0.65	0.65	0.66	0.66	0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.70	δ-BHC
0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.82	0.82	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.83	0.83	Heptachlor
0.89	0.89	0.88	0.88	0.87	0.87	0.87	0.86	0.86	0.85	0.85	0.85	0.84	0.84	0.83	0.83	0.83	0.82	2,4,5-T (IPE)
0.98	0.97	0.96	0.95	0.95	0.94	0.93	0.93	0.92	0.91	0.91	0.90	0.89	0.88	0.88	0.87	0.86	0.86	2,4-D (BE) II
0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	Dichlone
0.96	0.95	0.94	0.94	0.93	0.93	0.92	0.91	0.91	0.90	0.89	0.89	0.88	0.87	0.87	0.86	0.85	0.85	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.01	1.00	1.00	0.99	0.98	0.98	0.97	0.96	0.96	0.95	0.94	0.94	0.93	0.92	0.92	0.91	0.90	0.90	Ronnel
1.04	1.04	1.04	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.02	1-Hydroxychlordene
1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	1.35	1.34	1.33	1.32	Methyl parathion
1.53	1.53	1.52	1.51	1.50	1.50	1.49	1.48	1.48	1.47	1.46	1.46	1.45	1.44	1.44	1.43	1.42	1.42	Heptachlor epoxide
1.70	1.68	1.66	1.64	1.63	1.61	1.59	1.57	1.55	1.53	1.51	1.49	1.48	1.46	1.44	1.42	1.40	1.38	Malathion
1.64	1.63	1.61	1.60	1.59	1.57	1.56	1.54	1.53	1.52	1.51	1.49	1.48	1.47	1.45	1.44	1.43	1.41	DCPA
1.53	1.52	1.51	1.50	1.49	1.48	1.46	1.46	1.44	1.43	1.42	1.41	1.39	1.38	1.37	1.36	1.35	1.34	Dyrene
1.67	1.66	1.65	1.63	1.62	1.60	1.59	1.57	1.56	1.55	1.53	1.52	1.50	1.49	1.47	1.46	1.45	1.43	o,p'-DDE
1.62	1.61	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.52	1.51	1.49	1.48	1.47	1.46	1.45	1.44	1.43	Chlorbenside
2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.94	1.91	1.89	1.87	1.85	1.83	1.80	1.78	1.76	1.74	1.72	Ethyl parathion
1.99	1.98	1.97	1.95	1.94	1.93	1.91	1.90	1.89	1.87	1.86	1.85	1.83	1.82	1.80	1.79	1.78	1.76	Endosulfan I
2.16	2.14	2.11	2.09	2.07	2.05	2.02	2.00	1.98	1.96	1.93	1.91	1.89	1.86	1.84	1.82	1.80	1.77	p,p'-DDE
2.27	2.25	2.22	2.19	2.16	2.13	2.10	2.07	2.04	2.01	1.98	1.96	1.93	1.90	1.87	1.84	1.81	1.78	DDA (ME)
2.27	2.25	2.23	2.21	2.19	2.16	2.14	2.12	2.10	2.07	2.05	2.03	2.01	1.99	1.96	1.94	1.92	1.90	Captan
2.22	2.20	2.18	2.16	2.14	2.12	2.10	2.08	2.06	2.04	2.02	1.99	1.97	1.95	1.93	1.91	1.89	1.87	Folpet
2.43	2.41	2.39	2.37	2.35	2.33	2.31	2.29	2.27	2.25	2.22	2.20	2.18	2.16	2.14	2.12	2.10	2.08	Dieldrin
2.43	2.40	2.37	2.33	2.30	2.27	2.24	2.20	2.17	2.14	2.11	2.07	2.04	2.01	1.97	1.94	1.91	1.87	Perthane
2.34	2.31	2.29	2.27	2.24	2.22	2.19	2.17	2.15	2.12	2.10	2.07	2.05	2.03	2.00	1.98	1.96	1.93	o,p'-DDD
3.02	2.97	2.93	2.88	2.84	2.80	2.76	2.73	2.68	2.64	2.60	2.56	2.52	2.47	2.43	2.39	2.35	2.31	o,p'-DDT
2.76	2.73	2.71	2.69	2.67	2.64	2.62	2.60	2.58	2.55	2.53	2.51	2.49	2.46	2.44	2.42	2.40	2.37	Endrin
2.97	2.94	2.91	2.89	2.86	2.83	2.80	2.78	2.75	2.72	2.69	2.67	2.64	2.61	2.59	2.56	2.53	2.50	Chlordecone
3.22	3.17	3.13	3.08	3.04	2.98	2.94	2.90	2.86	2.82	2.77	2.73	2.68	2.64	2.59	2.55	2.51	2.46	p,p'-DDD
3.19	3.16	3.13	3.10	3.07	3.04	3.00	2.97	2.94	2.91	2.88	2.85	2.81	2.78	2.75	2.72	2.69	2.66	Endosulfan II
4.08	4.02	3.96	3.89	3.81	3.76	3.68	3.60	3.53	3.47	3.40	3.32	3.27	3.20	3.13	3.05	2.98	2.90	Ethion
4.04	3.98	3.92	3.86	3.80	3.73	3.67	3.61	3.54	3.48	3.43	3.36	3.30	3.24	3.18	3.12	3.06	2.98	p,p'-DDT
4.08	4.02	3.96	3.90	3.83	3.78	3.72	3.66	3.59	3.52	3.47	3.40	3.34	3.28	3.22	3.16	3.10	3.03	Carbophenothion
6.2	6.1	5.98	5.83	5.72	5.60	5.49	5.38	5.27	5.17	5.02	4.90	4.79	4.68	4.56	4.43	4.32	4.20	Dilan I
6.1	6.0	5.96	5.87	5.78	5.68	5.60	5.52	5.43	5.33	5.24	5.15	5.06	4.97	4.88	4.79	4.70	4.62	Mirex
6.7	6.5	6.4	6.3	6.1	5.98	5.84	5.70	5.57	5.42	5.29	5.16	5.01	4.89	4.73	4.60	4.46	4.32	Methoxychlor
7.4	7.2	7.1	6.9	6.8	6.6	6.5	6.3	6.2	6.0	5.87	5.71	5.57	5.41	5.26	5.11	4.96	4.81	Dilan II
11.6	11.3	11.1	10.8	10.6	10.3	10.1	9.8	9.6	9.3	9.0	8.8	8.5	8.3	8.0	7.8	7.5	7.3	Tetradifon
12.5	12.2	11.9	11.7	11.4	11.1	10.8	10.6	10.3	10.0	9.7	9.4	9.2	8.9	8.6	8.4	8.1	7.8	Azinphosmethyl
170		174		178		182		186		190		194		198		202	204	

Table 5. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 5% DC-200/ 7.5% QF-1 on 80–100 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 120 ml/min

170	174	178	182	186	190	194	198	202	204	
0.29	0.29	0.30	0.30	0.30	0.30	0.30	0.31	0.31	0.31	Mevinphos
0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.42	0.42	0.42	2,4-D (ME)
0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	Phorate
0.43	0.44	0.44	0.45	0.45	0.46	0.47	0.47	0.48	0.49	α-BHC
0.45	0.45	0.46	0.46	0.47	0.48	0.48	0.48	0.49	0.50	CDEC
0.54	0.54	0.54	0.55	0.55	0.55	0.55	0.55	0.55	0.56	2,4-D (IPE)
0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	Simazine
0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.57	0.57	0.57	Atrazine
0.59	0.59	0.58	0.58	0.58	0.58	0.57	0.57	0.57	0.57	Diazinon
0.56	0.56	0.56	0.57	0.57	0.58	0.59	0.59	0.60	0.61	Lindane
0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.65	0.65	0.65	2,4,5-T (ME)
0.57	0.58	0.58	0.58	0.58	0.59	0.59	0.60	0.60	0.60	β-BHC
0.83	0.83	0.82	0.82	0.81	0.80	0.80	0.79	0.78	0.77	2,4-D (BE) I
0.65	0.66	0.66	0.66	0.67	0.67	0.68	0.68	0.69	0.70	δ-BHC
0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.82	0.82	0.82	Heptachlor
0.90	0.89	0.89	0.88	0.87	0.86	0.85	0.84	0.83	0.82	2,4,5-T (IPE)
0.98	0.97	0.96	0.95	0.94	0.93	0.92	0.91	0.90	0.89	2,4-D (BE) II
0.84	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	Dichlone
0.95	0.94	0.93	0.93	0.92	0.92	0.91	0.91	0.90	0.89	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.02	1.01	1.01	1.00	1.00	0.99	0.99	0.98	0.96	0.94	Ronnel
1.27	1.25	1.24	1.23	1.22	1.21	1.20	1.19	1.18	1.17	1-Hydroxychlordene
1.54	1.53	1.51	1.51	1.49	1.49	1.47	1.46	1.45	1.44	Methyl parathion
1.54	1.53	1.53	1.52	1.51	1.51	1.50	1.49	1.49	1.48	Heptachlor epoxide
1.73	1.71	1.69	1.67	1.65	1.63	1.61	1.59	1.57	1.55	Malathion
1.70	1.68	1.67	1.65	1.63	1.62	1.60	1.59	1.57	1.55	DCPA
1.54	1.53	1.51	1.50	1.49	1.48	1.47	1.46	1.45	1.44	Dyrene
1.66	1.65	1.63	1.62	1.61	1.59	1.58	1.57	1.55	1.54	o,p'-DDE
1.62	1.61	1.60	1.59	1.58	1.57	1.56	1.55	1.54	1.53	Chlorbenside
2.14	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	1.95	Ethyl parathion
2.00	1.99	1.97	1.96	1.95	1.94	1.93	1.91	1.90	1.89	Endosulfan I
2.15	2.13	2.11	2.09	2.07	2.05	2.03	2.01	1.99	1.97	p-p'-DDE
2.28	2.25	2.22	2.19	2.16	2.13	2.10	2.07	2.04	2.01	DDA (ME)
2.35	2.33	2.31	2.28	2.26	2.23	2.21	2.19	2.17	2.14	Captan
2.24	2.23	2.21	2.19	2.17	2.15	2.13	2.12	2.10	2.08	Folpet
2.40	2.38	2.37	2.35	2.33	2.32	2.30	2.29	2.27	2.25	Dieldrin
2.42	2.39	2.36	2.33	2.30	2.27	2.24	2.21	2.18	2.15	Perthane
2.37	2.35	2.33	2.30	2.28	2.25	2.23	2.21	2.18	2.16	o,p'-DDD
2.91	2.87	2.84	2.81	2.78	2.75	2.72	2.69	2.65	2.62	o,p'-DDT
2.81	2.79	2.76	2.74	2.72	2.69	2.67	2.65	2.62	2.60	Endrin
2.96	2.93	2.91	2.89	2.86	2.84	2.81	2.79	2.76	2.74	Chlordecone
3.22	3.18	3.14	3.10	3.06	3.02	2.98	2.94	2.90	2.86	p,p'-DDD
3.22	3.19	3.16	3.13	3.11	3.07	3.05	3.02	2.99	2.96	Endosulfan II
4.10	4.04	3.98	3.91	3.85	3.79	3.73	3.66	3.60	3.54	Ethion
4.07	4.00	3.93	3.86	3.79	3.72	3.65	3.58	3.51	3.44	p,p'-DDT
4.12	4.06	4.00	3.94	3.88	3.82	3.76	3.70	3.63	3.57	Carbophenothion
6.3	6.2	6.1	5.98	5.87	5.76	5.65	5.53	5.42	5.31	Dilan I
5.98	5.91	5.83	5.76	5.68	5.61	5.53	5.46	5.38	5.30	Mirex
6.6	6.5	6.4	6.2	6.1	5.97	5.83	5.71	5.58	5.43	Methoxychlor
7.5	7.3	7.2	7.0	6.9	6.8	6.6	6.5	6.4	6.2	Dilan II
11.8	11.4	11.2	10.9	10.7	10.4	10.2	10.0	9.7	9.5	Tetradifon
12.5	12.2	12.0	11.7	11.4	11.2	10.9	10.6	10.4	10.1	Azinphosmethyl

170 174 178 182 186 190 194 198 202 204

Table 6. Retention ratios, relative to aldrin, of 51 pesticides and metabolites on a column of 1.6% OV-17/ 6.4% OV-210 on 80–100 mesh Chromosorb W (HP) at temperatures from 170 to 204°C; electron capture detector, tritium source, parallel plate; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml/min

170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	
0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.39	Mevinphos
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.51	2,4-D (ME)
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.54	Phorate
0.49	0.50	0.50	0.51	0.52	0.52	0.52	0.53	0.53	0.54	0.54	0.55	0.55	0.56	0.57	0.57	0.58	0.58	α-BHC
0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.57	0.58	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.59	0.60	CDEC
0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	2,4-D (IPE)
0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	Simazine
0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	Atrazine
0.71	0.70	0.70	0.69	0.69	0.69	0.68	0.68	0.67	0.67	0.66	0.66	0.65	0.65	0.64	0.64	0.64	0.63	Diazinon
0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.71	0.71	0.71	0.71	Lindane
0.84	0.84	0.83	0.83	0.82	0.82	0.82	0.81	0.81	0.80	0.80	0.80	0.79	0.79	0.78	0.78	0.78	0.77	2,4,5-T (ME)
0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	β-BHC
0.96	0.96	0.95	0.95	0.94	0.93	0.93	0.92	0.91	0.91	0.90	0.90	0.89	0.88	0.88	0.87	0.86	0.86	2,4-D (BE) I
0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	γ-BHC
0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	Heptachlor
1.02	1.02	1.01	1.01	1.00	1.00	0.99	0.99	0.98	0.98	0.98	0.97	0.96	0.96	0.96	0.95	0.95	0.94	2,4,5-T (IPE)
1.13	1.12	1.12	1.11	1.10	1.09	1.08	1.07	1.06	1.05	1.04	1.04	1.03	1.02	1.01	1.00	0.99	0.98	2,4-D (BE) II
1.14	1.14	1.14	1.14	1.14	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.12	1.12	1.12	1.12	1.12	1.12	Dichlone
1.30	1.29	1.29	1.28	1.28	1.27	1.26	1.26	1.25	1.25	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	Dimethoate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Aldrin (REFERENCE)
1.24	1.23	1.22	1.22	1.21	1.20	1.20	1.19	1.18	1.17	1.17	1.16	1.15	1.15	1.14	1.13	1.13	1.12	Ronnel
1.43	1.42	1.41	1.41	1.40	1.40	1.39	1.38	1.38	1.37	1.36	1.36	1.35	1.34	1.34	1.33	1.33	1.32	1-Hydroxychlordane
2.02	2.01	1.99	1.98	1.96	1.95	1.93	1.92	1.90	1.88	1.87	1.85	1.84	1.82	1.81	1.79	1.78	1.76	Methyl parathion
1.81	1.79	1.78	1.77	1.76	1.75	1.73	1.72	1.71	1.70	1.69	1.68	1.66	1.65	1.64	1.63	1.62	1.60	Heptachlor epoxide
2.37	2.33	2.30	2.26	2.22	2.18	2.15	2.11	2.07	2.04	2.00	1.96	1.93	1.89	1.86	1.82	1.78	1.74	Malathion
2.06	2.04	2.02	1.99	1.97	1.95	1.93	1.91	1.88	1.86	1.84	1.82	1.80	1.77	1.75	1.73	1.71	1.69	DCPA
2.14	2.12	2.09	2.07	2.04	2.02	2.00	1.97	1.95	1.92	1.90	1.87	1.85	1.83	1.80	1.78	1.76	1.73	Dyrene
2.04	2.02	2.00	1.98	1.95	1.93	1.91	1.89	1.87	1.85	1.83	1.80	1.78	1.76	1.74	1.72	1.70	1.68	o,p'-DDE
2.18	2.16	2.14	2.11	2.09	2.07	2.04	2.02	2.00	1.97	1.95	1.93	1.90	1.88	1.86	1.84	1.81	1.79	Chlorbenside
2.80	2.76	2.73	2.69	2.66	2.62	2.59	2.55	2.52	2.48	2.44	2.41	2.37	2.34	2.30	2.27	2.23	2.20	Ethyl parathion
2.33	2.31	2.29	2.27	2.25	2.23	2.21	2.19	2.18	2.16	2.14	2.12	2.10	2.08	2.07	2.05	2.03	2.01	Endosulfan I
2.67	2.64	2.60	2.57	2.53	2.49	2.46	2.42	2.39	2.35	2.32	2.28	2.24	2.21	2.17	2.14	2.10	2.06	p,p'-DDE
3.07	3.02	2.97	2.92	2.87	2.82	2.78	2.73	2.68	2.63	2.58	2.54	2.48	2.44	2.39	2.34	2.29	2.24	DDA (ME)
3.58	3.54	3.49	3.44	3.40	3.35	3.30	3.26	3.21	3.17	3.12	3.07	3.03	2.98	2.94	2.89	2.84	2.80	Captan
3.51	3.46	3.42	3.37	3.33	3.28	3.24	3.20	3.15	3.10	3.06	3.02	2.97	2.93	2.88	2.84	2.79	2.75	Folpet
2.94	2.92	2.89	2.86	2.83	2.80	2.77	2.74	2.71	2.68	2.65	2.62	2.60	2.57	2.54	2.51	2.48	2.45	Dieldrin
3.24	3.18	3.12	3.07	3.01	2.95	2.90	2.84	2.78	2.72	2.66	2.61	2.55	2.49	2.44	2.38	2.32	2.26	Perthane
3.26	3.20	3.16	3.10	3.05	3.00	2.96	2.90	2.86	2.80	2.76	2.71	2.66	2.61	2.56	2.51	2.46	2.40	o,p'-DDD
3.81	3.75	3.70	3.64	3.58	3.52	3.46	3.40	3.35	3.28	3.23	3.17	3.11	3.05	3.00	2.94	2.88	2.82	o,p'-DDT
3.68	3.64	3.59	3.54	3.50	3.45	3.40	3.36	3.32	3.27	3.22	3.18	3.13	3.09	3.04	2.99	2.95	2.90	Endrin
3.18	3.15	3.12	3.09	3.06	3.03	3.00	2.97	2.94	2.91	2.88	2.85	2.82	2.80	2.77	2.74	2.71	2.68	Chlordecone
4.63	4.55	4.46	4.38	4.30	4.21	4.13	4.05	3.96	3.88	3.80	3.72	3.63	3.55	3.46	3.38	3.30	3.22	p,p'-DDD
4.60	4.54	4.48	4.42	4.35	4.30	4.24	4.17	4.12	4.05	4.00	3.94	3.88	3.82	3.75	3.69	3.63	3.57	Endosulfan II
6.2	6.0	5.92	5.79	5.67	5.54	5.42	5.29	5.16	5.04	4.92	4.79	4.67	4.54	4.42	4.29	4.17	4.04	Ethion
5.46	5.36	5.26	5.16	5.06	4.96	4.86	4.76	4.66	4.56	4.46	4.36	4.26	4.16	4.06	3.96	3.86	3.76	p,p'-DDT
6.35	6.2	6.1	5.96	5.83	5.69	5.56	5.43	5.30	5.16	5.03	4.90	4.77	4.63	4.50	4.37	4.24	4.10	Carbophenothion
9.1	8.9	8.7	8.5	8.3	8.2	8.0	7.8	7.6	7.4	7.2	7.0	6.8	6.7	6.5	6.3	6.1	5.90	Dilan I
7.0	6.9	6.8	6.7	6.6	6.5	6.4	6.3	6.2	6.1	5.97	5.87	5.77	5.67	5.57	5.48	5.38	5.28	Mirex
10.9	10.6	10.4	10.2	10.0	9.7	9.4	9.2	9.0	8.8	8.5	8.3	8.0	7.8	7.6	7.3	7.1	6.8	Methoxychlor
10.5	10.3	10.0	9.8	9.6	9.4	9.1	8.9	8.7	8.4	8.2	8.0	7.8	7.5	7.3	7.1	6.8	6.6	Dilan II
17.5	17.2	16.8	16.4	16.0	15.7	15.3	14.9	14.5	14.1	13.8	13.4	13.0	12.6	12.3	11.9	11.5	11.1	Tetradifon
24.5	23.8	23.2	22.5	21.9	21.3	20.7	20.0	19.4	18.8	18.2	17.6	17.0	16.3	15.7	15.1	14.5	13.8	Azinphosmethyl
170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	200	202	204	

Table 7. Retention ratios, relative to ethyl parathion, of 54 organophosphorus pesticides and metabolites on a column of 10% OV-210 on 100–120 mesh Gas-Chrom Q at temperatures from 170 to 204°C; flame photometric detector, 526 nm filter; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml/min

170		174		178		182		186		190		194		198		202	204	
0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	TEPP
0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	Dichlorvos
0.12	0.12	0.12	0.12	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.14	Demeton thiono
0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.19	Mevinphos
0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	0.19	Thionazin
0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	Phorate
0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	Ethoprop
0.17	0.17	0.18	0.18	0.18	0.18	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21	Diazinon
0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.22	0.22	0.23	Sulfotepp
0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	Naled
0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	Oxydemeton methyl
0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	Disulfoton
0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.31	Dioxathion
0.29	0.29	0.29	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.31	0.31	0.32	0.32	Demeton thiolo
0.29	0.29	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.34	0.35	Dichlofenthion
0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.36	Diazoxon
0.34	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.39	0.40	Ronnel
0.40	0.40	0.41	0.41	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.42	0.43	0.43	0.43	0.43	0.43	0.43	Chlorpyrifos
0.43	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	Zytron
0.47	0.47	0.47	0.47	0.47	0.47	0.48	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.50	0.50	Bromophos
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	Fenthion
0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	Dimethoate
0.51	0.51	0.51	0.52	0.52	0.52	0.53	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	0.55	Ronnoxon
0.53	0.53	0.54	0.54	0.54	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.58	0.59	0.59	Cyanox
0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	0.61	Bromophos ethyl
0.67	0.67	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.64	0.64	0.64	0.64	0.64	0.63	Monocrotophos
0.75	0.75	0.75	0.74	0.74	0.73	0.73	0.73	0.72	0.72	0.71	0.71	0.71	0.70	0.70	0.70	0.69	0.69	Malathion
0.69	0.69	0.69	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.71	0.71	0.71	0.71	0.71	Iodofenphos
0.77	0.77	0.77	0.77	0.77	0.77	0.76	0.76	0.76	0.76	0.76	0.75	0.75	0.75	0.75	0.75	0.75	0.74	DEF
0.75	0.75	0.75	0.75	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.75	0.75	0.75	Phenthoate
0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	Folex
0.74	0.74	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	0.77	0.77	0.77	0.77	0.78	0.78	0.78	0.79	Methyl parathion
0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	0.81	0.81	Schradan
0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.86	0.86	0.86	Fenitrothion
1.02	1.01	1.01	1.00	0.99	0.99	0.98	0.97	0.96	0.96	0.95	0.94	0.94	0.93	0.92	0.92	0.91	0.90	Malaoxon
0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	Dicapthon
1.02	1.02	1.02	1.01	1.01	1.00	1.00	1.00	0.99	0.99	0.99	0.98	0.98	0.98	0.97	0.97	0.97	0.96	Crufomate
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Ethyl parathion (REFERENCE)
1.04	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	0.99	Methyl paraoxon
1.03	1.03	1.03	1.03	1.03	1.02	1.02	1.02	1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.01	1.00	Amidithion
1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.12	1.12	1.12	1.12	1.12	1.12	1.12	Methidathion
1.22	1.22	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.13	1.12	Tetrachlorvinphos
1.27	1.27	1.26	1.26	1.25	1.25	1.24	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	1.20	1.19	Carbophenothion
1.38	1.37	1.36	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.29	1.28	1.27	1.26	1.26	1.25	1.24	Phosphamidon
1.42	1.41	1.40	1.39	1.38	1.37	1.37	1.36	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.29	1.28	1.27	Ethyl paraoxon
1.43	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.37	1.36	1.35	1.34	1.33	1.32	1.31	1.31	1.30	1.29	Ethion
1.64	1.63	1.62	1.62	1.61	1.60	1.59	1.58	1.57	1.56	1.55	1.54	1.53	1.53	1.52	1.51	1.50	1.49	Carbophenoxon
2.09	2.08	2.06	2.04	2.02	2.00	1.98	1.96	1.94	1.92	1.90	1.88	1.86	1.84	1.82	1.80	1.79	1.77	Phenkapton
4.26	4.21	4.16	4.11	4.06	4.01	3.97	3.92	3.87	3.82	3.77	3.72	3.68	3.63	3.58	3.53	3.48	3.43	Fensulfothion
4.37	4.32	4.27	4.22	4.18	4.13	4.08	4.03	3.98	3.93	3.88	3.83	3.78	3.73	3.68	3.63	3.58	3.53	EPN
4.65	4.59	4.54	4.48	4.43	4.37	4.32	4.26	4.21	4.15	4.10	4.04	3.99	3.94	3.88	3.86	3.77	3.72	Imidan
6.98	6.88	6.77	6.66	6.55	6.44	6.33	6.22	6.11	6.00	5.89	5.78	5.67	5.56	5.45	5.34	5.24	5.13	Azinphosmethyl
6.86	6.76	6.66	6.56	6.47	6.37	6.27	6.17	6.08	5.98	5.88	5.79	5.69	5.59	5.49	5.40	5.30	5.20	Famphur
7.01	6.90	6.79	6.69	6.58	6.47	6.36	6.25	6.14	6.03	5.92	5.81	5.70	5.59	5.48	5.37	5.26	5.15	Azinphosethyl
19.1	18.7	18.3	17.9	17.5	17.1	16.7	16.3	15.9	15.5	15.1	14.7	14.3	13.9	13.5	13.1	12.7	12.3	Coumaphos
170		174		178		182		186		190		194		198		202	204	

Table 8. Retention ratios, relative to ethyl parathion, of 54 organophosphorus pesticides and metabolites on a column of 1.5% OV-17/1.95% OV-210 on 100–120 mesh Gas-Chrom Q at temperatures from 170 to 204°C; flame photometric detector, 526 nm filter; all absolute retentions measured from injection point. Arrow indicates optimum column operating temperature with carrier flow at 70 ml/min

170		174		178		182		186		190		194		198		202	204	
0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.09	TEPP
0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.09	0.09	0.09	0.10	0.10	0.10	0.11	0.11	0.11	0.12	Dichlorvos
0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20	0.21	0.21	Mevinphos
0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.21	0.21	0.21	0.22	Demeton thiono
0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.25	0.25	0.25	Thionazin
0.20	0.20	0.20	0.21	0.21	0.22	0.22	0.22	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.26	0.26	0.26	Ethoprop
0.23	0.23	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.29	0.29	0.29	0.30	Phorate
0.24	0.25	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.30	Sulfotepp
0.26	0.27	0.27	0.28	0.28	0.28	0.29	0.29	0.30	0.30	0.30	0.31	0.31	0.32	0.32	0.32	0.33	0.33	Oxydemeton methyl
0.32	0.32	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.37	Diazinon
0.31	0.31	0.32	0.32	0.33	0.33	0.34	0.34	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.38	0.38	0.39	Naled
0.34	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.36	0.37	0.37	0.37	0.37	0.38	0.38	0.38	0.39	0.39	Demeton thiolo
0.36	0.36	0.37	0.37	0.38	0.38	0.38	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.42	0.42	0.42	0.43	Disulfoton
0.37	0.38	0.38	0.39	0.39	0.39	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.43	0.43	0.43	0.44	0.44	Dioxathion
0.38	0.39	0.39	0.39	0.40	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	0.44	Diazoxon
0.44	0.44	0.45	0.45	0.45	0.46	0.46	0.46	0.47	0.47	0.47	0.48	0.48	0.48	0.49	0.49	0.49	0.50	Dichlofenthion
0.51	0.51	0.51	0.52	0.52	0.53	0.53	0.53	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.57	0.57	0.57	Cyanox
0.53	0.53	0.53	0.54	0.54	0.54	0.55	0.55	0.55	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.58	Dimethoate
0.55	0.55	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	0.61	0.61	0.62	Ronnel
0.57	0.57	0.57	0.58	0.58	0.59	0.59	0.59	0.60	0.60	0.61	0.61	0.61	0.62	0.62	0.63	0.63	0.63	Ronnoxon
0.78	0.77	0.76	0.76	0.75	0.74	0.73	0.73	0.72	0.72	0.71	0.70	0.69	0.68	0.68	0.67	0.66	0.65	Monocrotophos
0.66	0.66	0.67	0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.71	Zytron
0.74	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	0.76	0.76	Chlorpyrifos
0.74	0.74	0.74	0.75	0.75	0.75	0.76	0.76	0.77	0.77	0.77	0.78	0.78	0.78	0.79	0.79	0.79	0.80	Methyl parathion
0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81	0.81	Methyl paraoxon
0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.90	0.89	0.89	0.89	0.88	0.88	0.87	0.87	0.87	0.86	Malaoxon
0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.9	0.91	0.90	0.90	0.90	0.90	0.90	0.89	0.89	0.89	0.89	Malathion
0.85	0.85	0.86	0.86	0.86	0.86	0.87	0.87	0.87	0.87	0.88	0.88	0.88	0.88	0.89	0.89	0.89	0.90	Bromophos
0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	0.90	Fenthion
0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.92	0.92	0.92	0.92	0.92	0.92	Fenitrothion
1.02	1.01	1.01	1.00	1.00	1.00	0.99	0.99	0.98	0.98	0.98	0.97	0.97	0.96	0.96	0.96	0.95	0.95	Phosphamidon
1.25	1.23	1.21	1.19	1.18	1.16	1.14	1.13	1.11	1.09	1.07	1.06	1.04	1.02	1.00	0.99	0.97	0.95	Schradan
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Ethyl parathion (REFERENCE)
1.06	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.04	1.04	1.04	1.04	1.04	1.04	Ethyl paraoxon
1.06	1.05	1.05	1.05	1.06	1.06	1.06	1.06	1.06	1.06	1.05	1.05	1.05	1.05	1.04	1.04	1.04	1.03	Dicapthon
1.12	1.12	1.12	1.11	1.11	1.11	1.11	1.11	1.11	1.10	1.10	1.10	1.10	1.10	1.09	1.09	1.09	1.09	Bromophos ethyl
1.23	1.22	1.22	1.22	1.21	1.20	1.20	1.19	1.19	1.19	1.19	1.19	1.20	1.20	1.20	1.20	1.20	1.20	Amidithion
1.40	1.39	1.38	1.38	1.37	1.36	1.35	1.34	1.33	1.32	1.32	1.31	1.30	1.29	1.28	1.27	1.26	1.25	Crufomate
1.36	1.35	1.35	1.34	1.34	1.33	1.33	1.32	1.32	1.31	1.31	1.30	1.30	1.29	1.29	1.28	1.27	1.27	Phenthoate
1.51	1.50	1.49	1.49	1.48	1.47	1.46	1.45	1.44	1.43	1.43	1.42	1.41	1.40	1.39	1.38	1.37	1.36	Folex
1.51	1.50	1.49	1.49	1.48	1.47	1.46	1.45	1.44	1.44	1.43	1.42	1.41	1.41	1.40	1.39	1.38	1.37	DEF
1.57	1.57	1.56	1.56	1.56	1.55	1.54	1.54	1.53	1.53	1.53	1.52	1.52	1.51	1.51	1.50	1.50	1.49	Iodofenphos
1.72	1.71	1.70	1.69	1.68	1.67	1.66	1.65	1.65	1.64	1.63	1.62	1.61	1.60	1.59	1.58	1.57	1.56	Tetrachlorvinphos
1.74	1.73	1.73	1.72	1.72	1.71	1.71	1.70	1.70	1.69	1.69	1.68	1.68	1.67	1.67	1.66	1.65	1.65	Methidathion
2.69	2.67	2.64	2.62	2.59	2.57	2.54	2.52	2.49	2.46	2.45	2.42	2.40	2.37	2.35	2.32	2.30	2.27	Carbophenoxon
2.88	2.85	2.82	2.79	2.76	2.73	2.70	2.67	2.64	2.61	2.58	2.55	2.52	2.49	2.46	2.43	2.40	2.37	Ethion
2.99	2.97	2.94	2.92	2.89	2.87	2.84	2.82	2.79	2.77	2.75	2.72	2.70	2.67	2.66	2.62	2.60	2.57	Carbophenothion
4.65	4.60	4.56	4.51	4.46	4.42	4.37	4.32	4.27	4.23	4.18	4.13	4.08	4.04	3.99	3.94	3.89	3.85	Fensulfothion
5.57	5.50	5.42	5.35	5.27	5.20	5.12	5.05	4.97	4.90	4.82	4.75	4.67	4.60	4.52	4.45	4.38	4.30	Phenkapton
6.07	5.99	5.90	5.82	5.74	5.65	5.57	5.49	5.40	5.32	5.24	5.15	5.07	4.99	4.90	4.82	4.74	4.65	Famphur
6.63	6.53	6.44	6.34	6.25	6.16	6.06	5.97	5.86	5.78	5.69	5.59	5.50	5.41	5.31	5.22	5.12	5.03	EPN
7.95	7.84	7.72	7.61	7.50	7.38	7.27	7.15	7.04	6.93	6.81	6.70	6.58	6.50	6.36	6.24	6.13	6.01	Imidan
10.9	10.7	10.6	10.4	10.3	10.1	10.0	9.8	9.7	9.5	9.3	9.1	8.9	8.7	8.5	8.3	8.1	7.9	Azinphosmethyl
14.4	14.1	13.9	13.6	13.3	13.0	12.8	12.5	12.2	11.9	11.7	11.4	11.1	10.9	10.6	10.3	10.1	9.8	Azinphosethyl
22.2	21.8	21.3	20.9	20.4	20.0	19.5	19.1	18.6	18.2	17.2	17.3	16.8	16.4	15.9	15.5	15.0	14.6	Coumaphos
170		174		178		182		186		190		194		198		202	204	

Table 9. Retention ratios, relative to parathion, of 54 organophosphorus pesticides and metabolites on a column of 4% SE-30/6% OV-210 on 80–100 mesh Gas-Chrom Q at temperatures from 170 to 204°C; flame photometric detector, 526 nm filter; all absolute retentions measured from injection point. Arrow indicates optimum operating temperature with carrier flow set at 75 ml/min

170		174		178		182		186		190		194		198	↓	202	204	
0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09	Dichlorvos
0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.07	0.07	TEPP
0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.18	0.18	0.18	Mevinphos
0.15	0.15	0.15	0.16	0.16	0.16	0.17	0.17	0.17	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.20	0.21	Demeton thiono
0.16	0.16	0.16	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.21	0.21	0.21	0.22	0.23	Thionazin
0.19	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.22	0.22	0.22	0.23	0.23	0.23	0.23	0.24	0.24	0.24	Ethoprop
0.20	0.21	0.21	0.21	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	Phorate
0.21	0.22	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.27	0.27	0.27	Sulfotepp
0.23	0.23	0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.27	0.28	Naled
0.23	0.24	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.29	Oxydemeton methyl
0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.30	0.30	0.30	0.30	0.31	0.31	0.31	0.31	Diazinon
0.30	0.30	0.30	0.31	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.35	Dioxathion
0.32	0.32	0.32	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.35	0.35	0.35	0.35	0.36	0.36	0.36	0.37	Demeton thiolo
0.30	0.31	0.31	0.32	0.32	0.33	0.33	0.33	0.34	0.34	0.35	0.35	0.35	0.36	0.36	0.37	0.37	0.37	Disulfoton
0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.40	0.40	0.40	Diazoxon
0.40	0.40	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.44	0.44	0.44	0.45	0.45	0.45	Dichlofenthion
0.46	0.46	0.46	0.46	0.47	0.47	0.47	0.47	0.47	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.49	Dimethoate
0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.50	0.50	0.50	0.50	0.51	0.51	0.51	0.51	0.52	0.52	0.52	Ronnel
0.51	0.51	0.51	0.51	0.52	0.52	0.52	0.52	0.53	0.53	0.53	0.54	0.54	0.54	0.54	0.55	0.55	0.55	Cyanox
0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.57	0.57	0.57	0.58	0.58	0.58	0.58	0.59	0.59	0.59	0.59	Ronnoxon
0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.61	0.61	0.61	Monocrotophos
0.58	0.59	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.61	0.61	0.61	0.62	0.62	0.62	0.62	Chlorpyrifos
0.57	0.58	0.58	0.58	0.59	0.59	0.59	0.59	0.60	0.60	0.60	0.61	0.61	0.61	0.61	0.62	0.62	0.62	Zytron
0.62	0.62	0.62	0.62	0.63	0.63	0.63	0.63	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.65	0.65	0.65	Fenthion
0.67	0.67	0.67	0.68	0.68	0.68	0.68	0.68	0.68	0.69	0.69	0.69	0.69	0.69	0.70	0.70	0.70	0.70	Malaoxon
0.72	0.72	0.72	0.73	0.73	0.73	0.73	0.74	0.74	0.74	0.75	0.75	0.75	0.75	0.76	0.76	0.76	0.76	Methyl parathion
0.81	0.81	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79	0.78	0.78	0.78	0.78	0.78	Malathion
0.86	0.86	0.85	0.85	0.85	0.85	0.84	0.84	0.84	0.84	0.85	0.85	0.85	0.85	0.86	0.86	0.86	0.86	Fenitrothion
0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.91	0.91	0.91	0.90	0.90	0.89	0.89	0.88	0.88	0.88	0.87	Bromophos
0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	Methyl paraoxon
0.93	0.93	0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	Phenthoate
0.91	0.91	0.91	0.91	0.91	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.91	0.91	0.91	0.91	0.91	0.91	Bromophos ethyl
0.85	0.84	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.83	0.84	0.86	0.87	0.88	0.89	0.90	0.92	0.93	Schradan
0.96	0.96	0.96	0.96	0.96	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.98	Dicapthon
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	Ethyl parathion (REFERENCE)
1.02	1.02	1.02	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	1.00	1.01	1.01	1.01	1.01	1.01	1.01	Amidithion
1.02	1.02	1.02	1.02	1.02	1.02	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	Iodofenphos
1.11	1.11	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.08	Crufomate
1.18	1.18	1.17	1.17	1.17	1.16	1.16	1.15	1.15	1.15	1.14	1.14	1.13	1.13	1.13	1.12	1.12	1.11	DEF
1.19	1.18	1.18	1.17	1.17	1.17	1.16	1.16	1.15	1.15	1.14	1.14	1.14	1.13	1.13	1.12	1.12	1.12	Phosphamidon
1.18	1.17	1.17	1.17	1.17	1.16	1.16	1.16	1.15	1.15	1.15	1.14	1.14	1.14	1.13	1.13	1.13	1.12	Folex
1.25	1.24	1.24	1.23	1.23	1.22	1.22	1.21	1.21	1.20	1.20	1.19	1.19	1.18	1.18	1.17	1.17	1.16	Ethyl paraoxon
1.23	1.22	1.22	1.22	1.22	1.22	1.21	1.21	1.21	1.21	1.20	1.20	1.20	1.20	1.19	1.19	1.19	1.19	Methidathion
1.37	1.36	1.36	1.35	1.35	1.34	1.33	1.33	1.32	1.32	1.31	1.31	1.30	1.30	1.29	1.29	1.28	1.28	Tetrachlorvinphos
1.87	1.85	1.84	1.83	1.82	1.81	1.80	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.70	1.69	1.68	1.67	Ethion
1.89	1.88	1.87	1.86	1.85	1.84	1.83	1.82	1.80	1.79	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.71	Carbophenoxon
1.89	1.88	1.87	1.86	1.85	1.84	1.83	1.82	1.80	1.79	1.78	1.77	1.76	1.75	1.74	1.73	1.72	1.71	Carbophenothion
3.18	3.15	3.12	3.09	3.06	3.03	3.00	2.98	2.95	2.92	2.89	2.86	2.83	2.80	2.77	2.74	2.72	2.69	Phenkapton
3.96	3.92	3.87	3.83	3.79	3.75	3.70	3.66	3.62	3.58	3.53	3.49	3.44	3.41	3.36	3.32	3.28	3.23	Fensulfothion
4.65	4.60	4.55	4.50	4.45	4.40	4.35	4.31	4.26	4.21	4.16	4.11	4.06	4.01	3.96	3.91	3.87	3.82	Imidan
4.66	4.61	4.56	4.51	4.46	4.41	4.36	4.31	4.26	4.21	4.16	4.11	4.06	4.00	3.95	3.90	3.85	3.80	EPN
5.67	5.63	5.59	5.55	5.51	5.47	5.42	5.38	5.34	5.30	5.26	5.21	5.17	5.13	5.09	5.05	5.01	4.96	Famphur
7.48	7.38	7.27	7.17	7.06	6.96	6.85	6.75	6.64	6.54	6.43	6.33	6.22	6.12	6.01	5.91	5.80	5.70	Azinphosethyl
7.53	7.42	7.32	7.21	7.10	6.99	6.89	6.78	6.67	6.57	6.46	6.35	6.24	6.14	6.03	5.92	5.81	5.71	Azinphosmethyl
12.4	12.0	11.7	11.3	11.0	10.6	10.3	10.0	9.7	9.4	9.1	8.8	8.5	8.2	7.9	7.6	7.3	7.1	Coumaphos
170		174		178		182		186		190		194		198		202	204	

is included to demonstrate this. Actual RRT determinations were made for 8 chlorinated compounds at 5° intervals, using a column of 1.5% OV-17/1.95% QF-1 on 100–120 mesh Chromosorb W (HP).

Proceeding on this information, the RRT values for all compounds were determined at 3 temperature points, 170, 185, and 200°C. Three injections of each of the multicompound mixtures were made consecutively and the RRT_A or RRT_P values were calculated for each compound. The 3 values per compound were averaged and the resulting value was used as the plotting point for construction of the slope curve. Aldrin was used as the reference compound for all electron capture chromatography (RRT_A), and ethyl parathion was used for flame photometric chromatography (RRT_P).

During each change of oven temperature, from 200 to 185°C, and from 185 to 170°C, the calibration procedure was repeated. During the course of each run at a given temperature, the

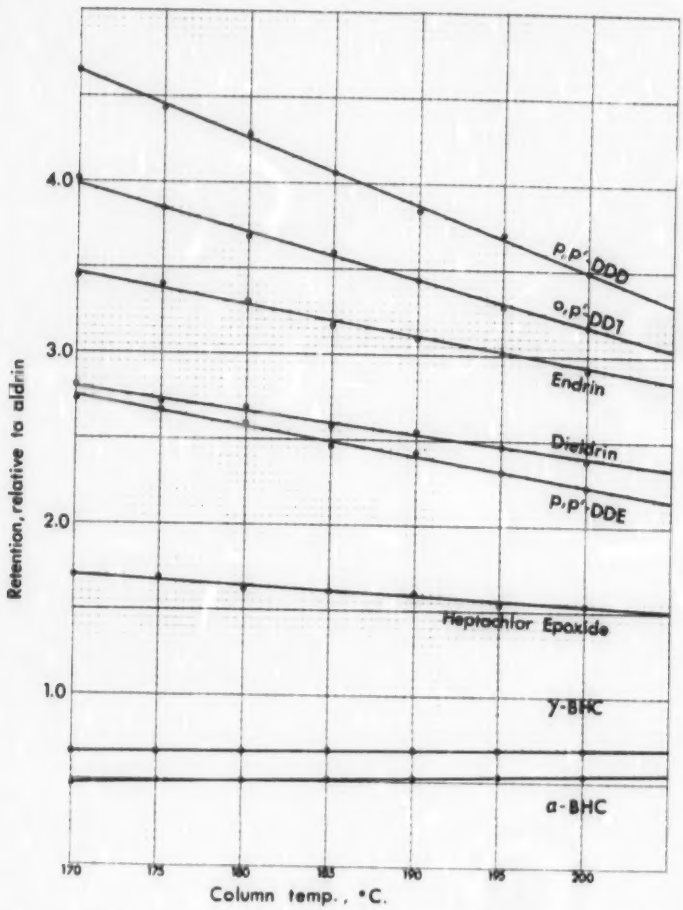

FIG. 1—Curves of 8 pesticidal compounds, plotting retention times, relative to aldrin, against column temperature at 5° intervals from 170 to 200°C. Column of 1.5% OV-17/1.95% QF-1 on 100–120 mesh Chromosorb W (HP).

oven temperature, as indicated by mercury thermometer and pyrometer, was constantly monitored.

Upon completion of all GLC work, a slope curve program was written for the computer. This program solved the equation of the regression line which provided the "best fit" to a set of x and y data points. The "normal equations" for a least squares line were solved simultaneously.

$$\Sigma y = bn + m\Sigma x \quad (1)$$

$$\Sigma xy = b\Sigma x + m\Sigma x^2 \quad (2)$$

obtaining

$$m = (n\Sigma xy - \Sigma x\Sigma y)/(n\Sigma x^2 - (\Sigma x)^2) \quad (3)$$

where n = the number of data points, each point consisting of a temperature (x) and a corresponding RRT (y).
Then the m value was utilized in solving equation 1, $b = (\Sigma y - m\Sigma x)/n$, where b = numerical distance on the x axis from the y axis and m = slope of a line.

At the top of each table, an arrow is placed over the temperature judged by the authors to be optimum for general usage. The criterion for these selections consisted of a compromise of the following factors: column efficiency, practicality of retention time in terms of work output, and resolution of the greatest number of pesticidal compounds.

Several compounds in the organophosphorus class exhibited multiple peaks via flame photometric detection on the 3 columns used, presumably resulting from the presence of impurities or decomposition products. These were naled, dioxathion, oxydemetonmethyl, azinphosethyl, carbophenoxon, Imidan, malaoxon, and fensulfothion. In each case the major peak was used for the RRT_P value.

The tables have proved very useful, particularly for tentative peak identifications in the chromatograms of multiresidue samples. First, it is necessary for the operator to establish the prevalent true column temperature. This is determined by chromatographing a standard mixture comprised of the appropriate reference compound (aldrin or parathion) and a relatively late eluting compound such as *p,p'*-DDT or Imidan. The $RRT_{A \text{ or } P}$ of the late eluting compound is calculated from the chromatogram. Then, by scanning horizontally across the column opposite *p,p'*-DDT (or Imidan) on the table, the RRT value is located which most closely matches the RRT value obtained by chromatography. The true column temperature can now be obtained by reference to the top or bottom of the table.

The sample extract is then chromatographed and the $RRT_{A \text{ or } P}$ values are calculated for all peaks appearing on the chromatogram. By vertically scanning the same temperature column on the table, the calculated RRT values may be compared with table values to obtain tentative peak identifications.

The system saves considerable time in that 2 chromatograms are generally sufficient to enable the operator to make tentative identifications of a number of the pesticidal compounds present in a sample. Using the more traditional approach, upon completion of the exploratory chromatogram on the sample extract, the operator usually selects reference standards on the basis of absolute retention values, or RRT values on the assumption that the column oven pyrometer readout is correct. If the actual oven temperature is significantly different from that indicated by the pyrometer, it may prove necessary to chromatograph a number of candidate reference standards before finding the compounds which produce comparable retention or RRT values.

Observations have occasionally been made that RRT_A values may shift slightly for certain organophosphorus compounds when they are chromatographed by electron capture detection on a given GLC column. Switching to an alternative column may result in an RRT_A value agreeing very closely with the table value. With this in mind, the chromatographer would be well advised to exercise caution in the identification of organophosphorus compounds determined by electron capture. Such compounds can be reliably identified by flame photometric detection as no such relative retention shifts have been observed in this detection mode when parathion has been used as the reference compound.

Conclusions

The data contained in the tables have proved very useful when coupled with the systematic approach described. The chromatographer is provided with a means of saving time and enhancing reliability in the tentative identification

of GLC peaks resulting from the injection of a substrate extract containing unknown levels of pesticide residues.

The analyst need not strive to precisely duplicate a specific column temperature given in a publication in order to match his relative retention values to those of the publication author(s). If one of the columns listed in Tables 1–9 is used, the analyst can determine his column temperature and then make tentative peak identifications from the RRT values given in the table.

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APPENDIX II

Environmental Protection Agency
Chamblee, Georgia 30341

Reproducibility of Micro-Mills Procedure for Pesticide Residues in Fat

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The size of the tissue specimen obtained at biopsy often severely limits the analytical chemist's study of the pesticide residue in the fat of the living person. For determination of chlorinated pesticide concentrations in small specimens, the micromodification of the Mills procedure is considered the method of choice. When interpreting results, however, knowledge is required of the quantitative differences in the procedure itself for each pesticide commonly found in fat, i.e., p,p′ isomers of DDT, DDE and DDD, dieldrin, benzene hexachloride (β-BHC) and heptachlor epoxide.

As an evaluation of the micro-Mills method in the examination of small specimens, two chemists, using different gas chromatographs in the same laboratory, analyzed quadruplicate aliquots of two specimens of human fat. Analytical results were examined statistically for each pesticide residue.

A specimen of panniculus adipose tissue was obtained at necropsy from two individuals and each specimen was divided into four pieces. Each piece was then divided into two aliquots which were analyzed separately by the two chemists on different days. Analysis of variance was used to determine the contribution of various factors to the variation among the measurement of each of six chemicals. Because of the experimental design, possible differences between the two chemists are compounded with differences between the two gas chromatographs differing in type of detectors.

The micro-Mills procedure described by Enos and his associates[1] was carried out as follows.

Approximately 170 to 250 mg of adipose tissue was extracted in a size B Duall tissue grinder with 2.3 ± 0.3 ml Nanograde® hexane and as a check for recovery, 20 ng of aldrin in 0.1 ml of hexane was added. The supernatant was poured into a 10 ml concentrator tube, and the extraction repeated. Extractions were combined in a 10 ml tube, concentrated to 500 microliters, and the concentrate volume was adjusted to 1 ml with Nanograde hexane that had been

previously saturated with Nanograde acetonitrile. The 2 ml of acetonitrile previously equilibrated with hexane was added and the solution placed in the Vortex mixer for two minutes. With a disposable pipette, the acetonitrile layer was transferred to a 25 ml test tube. Separation was repeated four times. The volume of hexane was maintained at 1 ml by adding hexane to the test tube each time the acetonitrile layer was transferred to the 25 ml test tube.

Twelve ml of 2% aqueous sodium sulfate and 2 ml of hexane was added to the test tube containing the combined acetonitrile extraction and was mixed thoroughly; then the hexane layer was transferred to a clean 10 ml concentrate tube. The extraction was repeated three times and concentrated to about 200 to 600 microliters.

After a florisil column had been removed from an oven heated to 130° C, allowed to cool to room temperature, prewetted with 10 ml of hexane and eluents discarded, the concentrate was transferred to the column with disposable pipette. (The column should not be allowed to become dry.) Collection of the eluate began immediately in a 25 ml tube. The elution was continued using 12 ml of hexane followed by 12 ml of 1% methanol (Nanograde) in hexane. These 24 ml represented the first fraction.

The second fraction was collected by eluting with 12 ml of 1% methanol in hexane in another 25 ml concentrator tube.

Both fractions were concentrated to 1 ml, and 5 microliters of each one injected into the gas chromatograph. The first fraction contained p,p′-DDT and p,p′-DDE (a small amount of βBHC and p,p′-DDD may appeár in the first fraction). The second contained p,p′-DDD, benzene hexachloride (β-BHC), heptachlor epoxide and dieldrin.

Two Micro-Tek-220 gas chromatographs were used: one, equipped with two nickel-63 detectors, operated at 253° C and 261° C (used by Chemist 2); the other, with tritium detectors, operated at 205° C and 210° C (used by Chemist 1). Each detector had two 6′ x ¼" glass tube columns. One contained 1.5% OV-17 and 1.95% QF-1 on 100/120 mesh Chromosorb-W.H.P.; the other, 4% SE-30 and 6% QF-1 on 80/100 mesh Chromosorb-W.H.P. Both were operated at 200° C.

Before measuring the test material, each machine was calibrated by three injections of the standard (5,6, and 7 μl) to check the linearity of the detector. Samples were diluted to bring a particular pesticide into the range of the standard. One fraction on one column could require as many as three injections of the test material (at

different dilutions) and as many injections of the standard to check on the sensitivity of the detector.

RESULTS

The levels of DDT, DDE, dieldrin and heptachlor epoxide were all recorded as measurable in these tissues. However, trace values which were below the limits of measurability, were recorded for 8 of the 16 DDD values and 1 of the 16 benzene hexachloride values. In order to have a number for computation, the arbitrary decision was made to use the number equal to half the value that would be measurable for that run.

The measurements obtained by each chemist were subjected to statistical analysis separately for each of the six residues. In the analysis of variance, the only significant differences were between levels of DDT, DDE, dieldrin and heptachlor epoxide in the two tissues. These results are shown in Table 1(A). There were no significant differences among measured levels in the separate pieces of the same tissue, none among levels because of chemist-chromatograph differences, or because of any interactions. Though the levels of the two tissues were significantly different, there was no significant difference in variability at these levels. Unexpectedly, it

TABLE 1. Significance of F-tests (Variance Ratios) from Analysis of Variance

	Pesticides					
	DDT	*DDE*	*DDD*	*Dieldrin*	*B-BHC*	*Heptachlor epoxide*
A. Diferences of Levels of Tissue A and Tissue B	<0.0005	<0.0005	NS*	<0.005	NS	0.005>p>0.001
B. Difference in reproducibility of Tissue A and Tissue B	NS	NS	NS	NS	NS	NS
C. Difference of instrument-chemist variability	NS	0.05>p>0.02	NS	NS	NS	0.01>p>0.001
D. Difference of Levels of instrument-chemists**	NS	NS	NS	NS	NS	NS

*NS = not significant

**would be significant if one chemist was consistently higher or lower than other chemist.

was noticed that there was greater variability among replicates processed by Chemist 1 on the chromatograph with the tritium detector than in those processed by Chemist 2 on the chromatograph with the nickel detectors, for DDE ($0.05 > p > 0.02$) and heptachlor epoxide ($0.01 > p > 0.002$) Table 1 (C).

Because of the difference in variability, the results obtained by each chemist are summarized separately in Table 2. The residues of DDT, DDE, DDD, dieldrin, benzene hexachloride, and heptachlor epoxide are expressed in parts per million (ppm) of wet tissue and the means of quadruplicate tests, range, standard deviation (s.d.) and coefficients of variations (c.v. = $\frac{\text{standard deviation)}}{\text{mean}}$ presented for each chemical.

The coefficient of variation was 11% or less for DDT, DDE, and dieldrin in each tissue. This is considered very satisfactory. It was 42% to 104% for DDD, 31% to 67% for benzene hexachloride, and 8% to 43% for heptachlor epoxide. Levels of DDD were admittedly at the lower limit of measurability, but the other two were believed to be measurable. The pooled standard deviation for each residue is presented in Table 2. This deviation reflects the variability of the measurement of the residue among all eight samples of each specimen.

Since the variability did not increase as the levels increased, it was considered valid to obtain a pooled standard deviation.

COMMENT

The 11% or less coefficient of variation for DDT, DDE and dieldrin in replicate analyses of tissues containing significantly different amounts of these chemicals lends credence to analytical results obtained by the micro-Mills procedure. Since the pooled standard deviation of DDT was 0.1326, of DDE 0.1605 and of dieldrin 0.0093, results for DDT or DDE should not be expressed to a precision greater than a tenth of a ppm. Magnitudes of dieldrin should be rounded to the nearest hundredth of a part per million.

For both chemists the coefficients of variation for DDD, benzene hexachloride and heptachlor epoxide at these levels were much greater than for DDT, DDE and dieldrin and were considered unsatisfactory. As for DDD, with half of the observations being arbitrary numbers substituted for traces, little can be concluded except that values should be rounded to the nearest hundredth of a ppm. Though the level of measurements of benzene hexachloride (<0.005 to 0.490 ppm) was similar to dieldrin (0.096 to 0.242 ppm),

TABLE 2. Pesticide Residue Analyses of Two Adipose Tissue Specimens in Quadruplicate by Each of Two Chemists, Expressed in ppm

Chemist	*Mean of Quadruplicates*	*Range*	*S.D.*	*Coefficient of variation S.D. $\bar{x}$*
A. p,p'-DDT - Tissue A				
1.	0.855	0.796- 0.962	0.074	9%
2.	0.845	0.822- 0.860	0.019	2%
p,p'-DDT - Tissue B				
1.	3.64	3.47 - 3.88	0.203	6%
2.	3.79	3.61 - 3.91	0.139	4%
	(pooled standard deviation 0.1326)			
B. p,p'-DDE - Tissue A				
1.	2.792	2.62 - 3.16	0.248	9%
2.	3.014	2.94 - 3.07	0.065	2%
p,p'-DDE - Tissue B				
1.	15.182	15.01 -15.26	0.117	1%
2.	15.200	15.08 -15.29	0.089	1%
	(pooled standard deviation 0.1605)			
C. p,p'DDD - Tissue A				
1.	0.006	<0.006- 0.011	0.003	53%
2.	0.010	<0.008- 0.018	0.007	67%
p,p'-DDD - Tissue B				
1.	0.012	<0.010- 0.030	0.012	104%
2.	0.008	<0.011-<0.014	0.003	42%
	(pooled standard deviation 0.0072)			
D. Dieldrin - Tissue A				
1.	0.110	0.096- 0.124	0.012	11%
2.	0.111	0.105- 0.116	0.006	5%
Dieldrin - Tissue B				
1.	0.228	0.210- 0.242	0.011	5%
2.	0.221	0.208- 0.227	0.009	4%
	(pooled standard deviation 0.0093)			
E. β BHC - Tissue A				
1.	0.271	<0.005- 0.391	0.182	67%
2.	0.310	0.209- 0.437	0.098	31%
β BHC - Tissue B				
1.	0.299	0.070- 0.388	0.153	51%
2.	0.315	0.051- 0.490	0.193	61%
	(pooled standard deviation 0.1497)			
F. Heptachlor epoxide - Tissue A				
1.	0.022	0.01 - 0.03	0.010	43%
2.	0.022	0.02 - 0.024	0.002	8%
Heptachlor epoxide - Tissue B				
1.	0.046	0.030- 0.055	0.011	24%
2.	0.033	0.031- 0.039	0.004	11%
	(pooled standard deviation 0.0084)			

the range of values was almost three times as great, the pooled standard deviation was 0.1497 and the coefficient of variation 37% to 67%; therefore, results should be rounded to the nearest tenth of a ppm. For heptachlor epoxide, a somewhat better result was obtained but it still was quite variable. Measurements of this chemical ranged from 0.01 ppm to 0.055 ppm with a pooled standard deviation of 0.0084 and a coefficient of variation, 8% to 43%. Levels should be rounded to the nearest hundredth of a ppm.

Since only two instruments, each operated by a different chemist, were used, it is impossible to do other than speculate as to the cause of the greater variability for measurements by chemist-chromatograph #1; it could be the performance of this one particular chromatograph; it could be the performance of tritium detector chromatographs of which this is a sample; or it could be a difference between two chemists. Only a well-designed blind study with replication of various chromatographs in several laboratories could give information as to the relative magnitude of different sources of variability among instruments and laboratories.

Although this unexpected difference in variability between instruments in measurement of DDE was observed to be significant ($p<0.05$), the magnitude of the difference was not deemed important; the highest coefficient of variation was less than 10% and the pooled standard deviation was 0.1605.

SUMMARY

The reproducibility of the micromodification of the Mills procedure for detection of six pesticide residues in biopsied adipose tissue was investigated. The magnitude of the pooled standard deviations implies that the measurements should be rounded to the nearest tenth of a ppm for DDT, DDE, and benzene hexachloride, and to the nearest hundredth of a ppm for DDD, dieldrin, and heptachlor epoxide.

Although variability among measurements of certain pesticides by a tritium detector chromatograph was found to be significantly greater than among measurements by a nickel detector chromatograph, no inferences can be made from this sample of only two chromatographs.

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